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THE BEARING OF SOME GENERAL BIOLOGICAL FACTS ON BUD-VARIATION[1]

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I TAKE it no one denies that in the Angiosperms variations may be produced in connection with reproduction by means of buds and that these variations may be perpetuated by the same method. Practically, as horticulturists and plant breeders, we care little about the occurrence of bud-variations elsewhere in the organic world. Nevertheless, it may help in the orientation of our ideas if we remember that budding is not a rare or unconventional method of reproduction. In a generalized form, the earliest method, it has persisted throughout the plant kingdom from the most primitive to the highest and most specialized types. Sexual reproduction has not replaced it, but has been added to it. Even in the animal kingdom, though eliminated among the higher forms, it still exists as an occasional alternate method in three fourths of the phyla. Such being the case, it would seem logically to follow that variation must have been within its possibilities.

The cause, the frequency, the type, the constancy, the mechanism, of these variations are more debatable, however, and on these questions many biological facts which superficially seem unconnected, have a direct bearing. In

[1] Read before the meeting of the Society for Horticultural Science, December 28, 1916.

fact, on certain phases circumstantial evidence is the only evidence at hand.

The exact nature of the cause or causes of bud-variation can hardly be discussed profitably. We may imagine irregularities of cell division directed by combinations of unknown factors, but to describe these factors in concrete terms is at present impossible. At the same time, cause can not be neglected entirely even at present, for cause in a generalized sense is intimately connected with frequency in that vigorous perennial the question of the inheritance of acquired characters. The data on this subject are so voluminous that each for himself must give them careful conscientious consideration. Here no more can be done than to point out some of the conclusions to which I, personally, have been driven, and their connection with the subject in hand. These conclusions are:

1. Broad and varied circumstantial evidence indicates unmistakably that the inheritance of acquired characters has played an extremely important rôle in evolution.

2. Numerous experimental investigations designed to test the possibility of such inheritance directly have either failed utterly or have been open to serious destructive criticism. Direct proof of the inheritance of acquired characters is therefore lacking.

3. If conclusions 1 and 2 are to be harmonized, either modifications are fully inherited so rarely that proof that they do not belong to the general category of chance changes in constitution of the germ-plasm is impossible, or the imprint of the environment is so weak that extremely long periods of time—perhaps geological epochs—are necessary for its manifestation.

Diametrically opposed views on the inheritance of acquired characters are held tenaciously and unequivocally by equally eminent biologists. Those who concur with the Lamarckian position are nearly always the students of evolution who approach the subject from the historical or the philosophical side and who rely almost entirely on circumstantial evidence; those who adhere to the side

of Weismann are usually experimentalists whose evidence is indeed direct, but often questionable, usually capable of various interpretations, and always fragmentary. I have been bold enough to grasp both horns of the dilemma, and to plead that each is right from his point of view. My confession of faith is, the environment has been an immense factor in organic evolution, but its effects are shown either so infrequently or after the elapse of so great a time, that for the practical purposes of plant breeding we can neglect it as we would neglect an infinitesimal in a calculation. As Bergson, I think, said:

> We have been trying to prove that the hour hand moves, in a second of time.

A few words will make clear the general arguments in favor of this position, although adequate support to the thesis would require considerable time.

In the first place, it seems to me the possibility of the inheritance of acquirements must be admitted. Weismann's general contention that the chromatin of the germ-cells is the actual hereditary substance, and that the germ-cells themselves may be regarded as one-celled organisms reproducing by fission and conjugating at certain times, while the body must be considered simply an appendage thrown off from and independent of the germ-cells, is not supported merely by the embryological researches of Boveri, Kahle and Hegner on two or three animal forms, or by the ingenious ovarian transplantations made by Castle and Phillips on guinea pigs, but by all of the recent pedigree culture and cytological genetic work, botanical as well as zoological. Nevertheless it has not been and logically can not be proven that there is no way for environmental forces to produce germ-plasmic changes. Memory is just as strange a phenomenon and Semon has done biology a service by pointing out the analogy between the mechanical requirements for memory and for the inheritance of somatic modifications.

This possibility being admitted, one may well concede the plausibility of the arguments of the numerous pale-

ontologists, taxonomists and ecologists in favor of Lamarckian principles, in spite of the fact that their evidence is circumstantial. They take a comprehensive view of the actual conditions that exist among organisms, which is impossible to the experimentalist. It will not do simply to say that the manifest convergence of analogous organs in all parts of the organic world, or the wonderful adaptations of the social insects *may be explained in some other way.* Of course there may be other explanations for these phenomena; but until more satisfactory explanations are forthcoming it is rightfully a custom in science that the adequate interpretation at hand should be accepted.

On the other hand it is equally wrong for the ardent devotees of Lamarckism to clutch at every isolated case, every inadequate and abortive experiment, when judicial consideration shows not a single *unassailable* instance of the inheritance of a somatic modification. Many of these experiments have a direct bearing on bud-variation, and I shall attempt to show where they lead us.

1. *Inheritance of Mutilations.*—The most radical Lamarckians of the present day only go so far as to suppose that mutilations are inherited on very rare occasions—and they are always zoologists. Ethnology has furnished us with so many histories of mutilations of ears, of lips, of feet, of reproductive organs, long continued in the folkways of a people, that new laboratory experiments have been deserving of the ridicule they have received. Botanists have seldom had any delusions on the subject. Plants are so continually mutilated in the buffetings they receive during life, with no result in the next generation, that the non-inheritance of the effects of such injuries is taken as a matter of course. Yet there is occasionally one whose reason fails at the critical moment, and who holds that cuttings from the chrysanthemum with the large flower resulting from the removal of lateral branches, will produce larger flowers in the next generation than will an untreated sister plant. If not this, some equally indefensible doctrine.

2. *Effects of Changed Food Supply.*—This last example was really one of changed food supply induced by mutilation. Change of food supply by other methods has been the basis of scores of experiments, particularly on insects. Many insects are so very whimsical about what they eat that it seems possible their selective appetite may be an inherited instinct impressed by the environment of countless generations. But the total result of all experiments on them is merely to prove that a second generation may be influenced in the start they get in life by the nutrition of the mother.

The same thing is true in plants. We fertilize a pop corn to get a bumper crop of good plump healthy seeds, but we don't expect a dent corn as the next year's result. We very properly endeavor to give our potatoes a balanced ration, in expectancy of a larger yield of well-matured, healthy tubers, but we should not expect these tubers to affect our next season's supply other than by their health. Similarly we take scions from well-lighted parts of the tree where growth has been good. In such twigs the graft union heals easily and properly, and a fit channel for conveying nutrients is established. In doing these things we are practising sanitation or preventive medicine, as it were, a laudable proceeding. But the horticulturist who promises a *different variety* by such means is illogical and misleading.

Yet we find Bailey so imbued with the idea of making out a perfect case for Lamarckism that he lends the weight of his authority to the following statement among others:[2]

> Whilst these "sports" are well known to horticulturists they are generally considered to be rare, but nothing can be farther from the truth. As a matter of fact, every branch of a tree is different from every other branch, and when the difference is sufficient to attract attention, or to have commercial value, it is propagated and called a "sport."

We may admit the differences between the branches of a tree without cavil. What is more serious is the impli-

[2] "Survival of the Unlike," p. 72.

cation to the reader that all variations have the same coefficients of heredity, that a bud-variation is simply a wide fluctuation imposed by external conditions. If this were true the whole organic world would be chaos. But species and varieties do exist. They may be "judgments" in one sense, but in another they are concrete things. In fact we learn this further on in this volume when it suits Bailey's purpose to have asexually propagated varieties very constant. He says (p. 353):

> At first thought this fact—that varieties may be self-sterile—looks strange, but it is after all what we should expect, because any variety of tree fruits, being propagated by buds, is really but a multiplication of one original plant, and all the trees which spring from this original are expected to reproduce its characters.

3. *The Effects of Disease.*—The influence of disease is in many ways like that of malnutrition, in that it is wholly an effect on the physiological efficiency of the reproducing cells. This fact is fairly clear when dealing with diseases with outstanding symptoms. In many instances, however, diseases are not easily diagnosed. There may even be no suspicion that disease is present. In such cases it is rather hard to believe that selection is not accomplishing a positive and radical improvement. A good example of this is the selection of potato tubers. No one consciously selects a seed potato infected with blight. Independent of the probability of reinfection, there is the likelihood that the diseased tuber will not be able to produce a normal plant because of the effect the fungus has had on its own cells. One doesn't usually believe, however, that rejection of this tuber and selection of the healthy sister is going to lead to the formation of a new race. Yet numerous experiments on potatoes in which it is shown that successive selections have raised the average yield over that of the unselected tubers, are probably of just this type. The race is kept up by the rejection of diseased tubers, but there is no evidence whatever that it is *improved*. I am not going to argue that desirable asexual variations may not occur during this time, and be retained. I say only that any improvement

indicated by the raw data must be discounted by the amount of deterioration shown by the unselected variety under similar conditions. Such deterioration is very common, and is due to disease, I believe, rather than to any supposed disadvantage of asexual reproduction *per se*.

This category of facts has been cited under the discussion of the inheritance of acquired characters, because such phenomena have perplexed other than botanists. Belief in the transmission of disease, or the effects of disease, by sexual reproduction was current for many years. It is only since the possibility of infection in the egg itself was demonstrated for various diseases, that the true state of affairs has been known.

Many other types of experiments designed to demonstrate Lamarckism might be cited, but they have no direct bearing on bud-variation except in so far as a positive case would affect our general attitude on the frequency of their occurrence. They are all similarly negative or questionable, however, so that we must conclude with Weismann that no case of inheritance of acquirements has been proved beyond a reasonable doubt. In other words we grant such a possibility but believe it to be so rare or so gradual that practically it may be disregarded.

In reality one could hardly have expected any other conclusion from the type of experiment by which the question has been attacked. Generalized they are something like this. Species *X* having been grown under environment *A* for numerous generations is removed to environment *B*. An adaptive change occurs which persists during several generations. Later the descendants of the original plants are returned to environment *A* and the change is reversed. When the reverse change occurs more slowly than the original change, it is argued that Lamarckian inheritance is shown. The logic used to draw such a conclusion is indefensible, even if the difficulty of correcting properly for changes due to normal heredity is left out of consideration.

If acquired characters are inherited and the changes

induced are reversible, the long period under environment *A* should have produced a deep impression on species *X*. Change under environment *B* should be slow. Reversal should be rapid, however, because of the slight impression environment *B* must be supposed to have made during the very few generations in which its influence was possible.

If acquired characters *are not inherited,* precisely the same changes should occur, owing to somatic adaptation, the only differences being that the total amount of change in each case would be reached in the second generation after the environment had acted during the earliest stages of the life history.

If, on the other hand, the changes induced by environment *B* are not reversible, judgment must be based on the percentage of individuals changed by *B* and not rechanged by *A*. One can readily see how a just judgment would be clouded by probable reversible somatic effects in such cases. Instances of the inheritance of acquirements, unless they were very frequent, which from our general evidence is unthinkable, would be indistinguishable from ordinary chance variations.

Such methods of attack on the subject being almost predestined to failure from the inherent difficulties of the problem, it would seem wiser to seek for a more hopeful methodology, and in the meantime to accept the only conclusion justified by the data at hand; namely, the inheritance of acquired characters is either so rare an occurrence or so slow a process, that by plant-breeders it may be assumed to be non-existent. One realizes of course that the problem of sexual transmission of somatic acquirements is not necessarily the same as that of asexual transmission, but the experimental results have been the same in both cases. Let us, admit, therefore, that one can not hope to obtain real improvement in asexually propagated varieties merely by selecting buds from plants or parts of plants which have developed under especially favorable conditions.

This does not mean that radical environmental changes

may not be the direct cause of such a modification. Dr. H. J. Webber once informed the writer that immediately after the great Florida freeze of the early nineties bud-variations in the citrus fruits of that region were greatly increased. Such variations may have been induced by the freezing, but they were not adaptive variations.

The conclusions reached thus far have not involved a point of theory which practically is difficult to separate from the one just discussed. It is this. If we disregard adaptive variations, is there not still a reason for selecting fluctuations? Are there not internal factors which so act that there is a narrow but appreciable variability in an asexually produced population which may offer a basis for selection? In other words, how constant is an asexually propagated race?

We can make an effort to compute the frequency of marked bud-variations. But have we any right to assume that these represent the sum total of all bud-variations? Are not bud-variations and perhaps all inherited variations like residual errors, the small ones frequent, the large ones rare? This may be the case, but I should like to emphasize the fact that we have no true criterion for determining the size of a variation. A variation that appears large by visual criteria may be an extremely small change in the constitution of the plant, and *vice versa.* In view of this fact together with the practical consideration that commercially valuable variations must be measurable within a reasonable duration of time—say a lifetime—it is by no means certain that we are going far astray in calculating the frequency of bud-variations by the so-called marked jumps or mutations.

Furthermore the range of the fluctuations of asexually propagated varieties of most species is very small even when broadened—as it always is—by the addition of the effects of variable external conditions. It is not hard to recognize a Winesap apple, a Clapp's Favorite pear or a Concord grape, even though these varieties have been grown extensively for a considerable number of years. Certain local subvarieties of the pome fruits are said to

exist, but they are so extremely rare that one may admit all cases of disputed origin and still have very little asexual variation to account for.

I have never seen a published calculation of the frequency of bud-variation, and presume it would be of little value anyway, since the general evidence indicates a different frequency for different species and even for the same species at different times. It may be mentioned, however, that in personal examination of over 100,000 hills of potatoes belonging to several hundred varieties, 12 definite bud-variations have been seen, a frequency of 1 in 10,000; while just as careful a scrutiny of about 200,000 plants belonging to the genus *Nicotiana* has brought to light but 1 case.

Probably a more practical and just as satisfactory an estimate of the frequency of bud-variations in economic plants is the record of varieties that have been produced in this manner. Naturally such a record contributes little to theory because only a portion of the variations arising are observed, and only a fraction of those observed are propagated. Further, the origin of comparatively few commercial varieties is known. Yet we may get some idea of what to expect in the future, by noting what has occurred in the past.

Data gathered in this manner will appear to give us different values depending on how we approach the matter. For example, in Cramer's wonderful monograph on bud-variation, the grape is cited as one of the species that often varies in this manner. He cites some 25 or more such varieties. Yet in the large list of American grapes in Hedrick's "Grapes of New York" only one doubtful case of bud-origin is reported. When one remembers that hundreds of varieties of grapes are grown and millions of vines are examined each year, improvement by this method seems rather hopeless. And examination of the list of present-day apples, pears, plums and cherries, of the bush-fruits, or of potatoes—all groups of considerable horticultural importance—is still more disappointing, for I venture to say that the varieties of

these types in cultivation which have originated as bud-variations can be counted on the fingers of one hand.

At the same time it would be wrong not to attribute any importance to bud-variation as a plant breeding adjunct. Cramer lists several hundred chrysanthemums and over a hundred roses as of bud-origin, as well as a smaller number of varieties in species where bud-variation appears to be less prevalent. Further, Shamel is said to have found bud-variation in the citrus-fruits to be sufficiently common to be worthy of an extended investigation.

These species, however, with perhaps the banana and the pineapple—the origin of whose varieties is little known—are the outstanding examples of comparatively frequent bud-variation, picked from our whole long list of cultivated plants. The first two examples, moreover, are species belonging to the domain of floriculture, where rather superficial characters such as color are valuable. In very few other species have bud-variations been recorded in sufficient numbers to justify us in employing any other adjective than "rare" in describing them. And of the sum total of these varieties only an extremely small percentage are of such a nature that agriculture would suffer a material loss if they were eliminated.

Perhaps these last statements appear to imply a very limited type of bud-variations. This is not true. Bud-variations are wholly comparable to seed-variations in their nature, but they are handicapped because recombinations of variant characters are possible only in sexual reproduction. N bud-variations in a species are simply N variations, but N seed-variations may become 2^n seed-variations provided they are not linked together in heredity. An immense advantage thus accrues in favor of seminal reproduction because by far the greater number of commercially valuable characters are complex in their heredity, *i. e.*, they are represented in the germ-plasm by several factors independently inherited.

Cramer divides bud-variations into the same classes that de Vries has used for sexual mutations: progressive,

where new characters arise; retrogressive, where a character becomes latent or lost; and degressive, where latent characters become active. In this important monograph practically all recorded bud-variations to the date of publication, 1907, are discussed. Yet not a single case of *progressive* variation is listed. They are all catalogued as retrogressive or degressive. Their classification is correct, however, only when a progressive variation is defined as the addition of a character wholly unknown in the previous history of the species.

As examples of what bud-variation does produce we may well study Cramer's painstaking work. There are losses of thorns, hairs and other epidermal characters, together with an occasional degressive change of the same kind. There are changes in color in vegetative parts. Green becomes red or "aurea" yellow, or a loss of anthocyan occurs. Sometimes the changes are such that the plants remain striped or otherwise variegated. Flowers and fruits exhibit the same types of color variations in considerable numbers. They are mostly losses, with the appearance of what in Mendelian terminology is called hypostatic colors, but once in a great while epistatic colors recur anew.

Monstrosities appear. Other parts of the flower take on the appearance and form of petals or of sepals. Doubling occurs in several different ways. Fasciations arise. Changes in the character of the reproductive apparatus are not uncommon, sometimes giving us seedless fruits.

Plants change their habit of growth. They become dwarf. They retain juvenile characters. They become laciniate, or develop the trait known as "weeping."

Thus we see that bud-variation is not limited in its manifestations; and what is more important, we realize that bud-variations are very comparable to seminal variations, there being hardly a type of change known in sexually reproduced plants that has not been duplicated asexually. What then is the difference, if any, between true somatic changes and true germinal changes in constitution? We can get clues which indicate a fairly satis-

factory solution of this problem from three different lines of research, pedigree cultures, graft-hybrids and cell-studies.

It is a noteworthy fact that the character of the progeny produced sexually by bud-variations has been studied in a comparatively few cases, and in most of these instances self-pollinations were not made. Nevertheless Cramer believes the following conclusions are justified:

1. In a vegetative Mendelization, of the progeny of a branch with the positive character 75 per cent. have the character and 25 per cent. are without it, while the progeny of a branch without the character all lack it.

2. In a vegetative "Zwischenrasse" by which he generally means a variegated race, of the progeny of each type (original and variant), a part retain and a part lack the character, the percentage being variable.

3. In a vegetative mutation, by which he means any change not a "Zwischenrasse" and which did not appear to him to be Mendelian in type, of the progeny of a branch retaining the positive character, either all possessed it or a part were with and a part without it, while the progeny of a branch without the character were all of the same type.

If we allow for some deviation due to cross-pollination, I believe that Cramer's records support this view, and that modern genetic research suggests the interpretation.

In the first place, the "Zwischenrasse" are evidently of the type studied principally by Correns and by Baur in sexually reproducing races. They are due to chromatophore changes, and in many cases at least are not the result of nuclear activity. This being true, one would expect in neither asexual nor sexual reproduction the same type of inheritance for variegated races that obtains for other types of variation. Inheritance will parallel cytoplasmic rather than nuclear distribution; an expectation apparently realized for both types of reproduction.

Omitting the "Zwischenrassen" therefore, we have two phenomena to explain, both of which are similar to cases of inheritance in sexual reproduction where chromatin

distribution parallels the facts. In each instance the negative variant—may we call it the recessive—breeds true. In one case the positive variant breeds true, in the other case it gives a simple Mendelian ratio.

The mechanism necessary for such phenomena is not difficult to picture. Bud-variations are many times more frequent in hybrids, that is, in plants heterozygous for one or more characters, than they are in *pure* species. This is the view of Cramer, this was the view of Masters, the eminent English student of bud-variations and teratological phenomena, this was the conclusion drawn by the present writer in several articles published some years ago. Such results would be obtained either when the proper germinal change occurs in the chromosome whose mate lacks a character for which the plant is heterozygous; or, when there is a dichotomy in which the chromosomes of such a pair are not halved but pass the material basis necessary for the production of the positive character to one daughter cell and not to the other, provided the daughter cell lacking the character gives rise to a branch.

A bud-variation in a character for which the plant was homozygous would be obtained only when simultaneous like changes occur in both chromosomes of a homologous pair, or when the material basis necessary for the production of the positive character all passes to one daughter cell, as described above.

This hypothesis would account for the fact that heterozygotes give rise to bud-variations more frequently than homozygotes, since a germinal change seldom gives rise to a new positive character, and a change in one chromosome of an identical pair tending toward the production of a recessive, would not show in the latter case.

I am not certain that this hypothesis may not with reason be applied to variations that are usually considered seminal. There is no particular ground for assuming that such variations occur only at the maturation of the germ-cells. We know that progressive variations of whatever origin are extremely rare. Why then may not

most variations be produced in cell divisions previous to the formation of the germ-cells? When recessive we should not note them as bud-variations unless the plant is heterozygous and the mutating cell gives rise to a branch; when dominant we should only note them in the latter eventuality. But if these mutating cells should later give rise to germ-cells, the change would become apparent in the progeny.

We have still one other hypothetical case to consider. It is said that some bud-variations are not transmitted by seed. I have not been able to trace an authentic case, but such is the general belief, fathered, I think, by Darwin. The usual citation is the nectarine, which sometimes is said to give nectarines but at other times gives only peaches. Whether trichome characters only behave thus I do not know. But if that be true, we can understand why if we refer to Winkler's work on the so-called graft-hybrids.

Winkler found that the most interesting of these peculiar phenomena are caused by the tissue of one species growing around the tissue of the other. He therefore gave them the euphonious name of periclinal chimeras. Cytological examination showed that the epidermal tissues only are from one race, the remaining tissues being from the other. It is really a symbiosis and not a union. Now as the germ-cells are formed wholly from subepidermal and never from epidermal tissues, the seeds of these plants always produced seedlings like the type forming the *inner cell-layers.*

It seems probable that the production of the nectarine may be analogous. If the change producing the nectarine occurs after the epidermal tissue has been segregated from other tissues, the cells which are ancestors of the germ-cells should not be affected and the nectarine seedlings would give peaches. If, on the other hand, the change producing the nectarine, has occurred before any such segregation, the progeny sexually produced should in part be nectarines.

THE PROBABLE ERROR OF A MENDELIAN CLASS FREQUENCY[1]

DR. RAYMOND PEARL

1. With the increasing volume of Mendelian experimentation there is an ever-growing need for adequate and clearly understood tests for the statistical significance of differences between observed results and expectation. A number of different methods of making such tests have been proposed and used by different workers. For example, early in the discussion of Mendelism, Weldon[2] made use of the ordinary, and frequently inadequate, expression for the standard deviation of a sub-frequency $\sigma = \sqrt{npq}$. Johannsen[3] has also made much use of this method. It has several defects. In the first place it assumes the Gaussian distribution of the errors, an assumption not often strictly warranted, as Pearson[4] has clearly shown, and in many cases grossly in error. In the second place it is not even approximately adequate under certain extreme conditions (frequently realized in Mendelian work) of class frequency. Harris[5] has proposed the χ^2 "goodness of fit" test for comparing observed and expected Mendelian distributions. There are several features of this method which greatly limit it for such use. Among these are (1) its failure to make correct allowance for "tail" frequencies (it is just this class of very small frequencies which one most often wants to test in prac-

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 108. The author is greatly indebted to his assistant, Mr. John Rice Miner, for the laborious arithmetic involved in Section 6 of the paper.

[2] Weldon, W. F. R., "Mendel's Laws of Alternative Inheritance in Peas," *Biometrika*, Vol. I, pp. 228–265, 1902.

[3] Johannsen, W., "Elemente der exakten Erblichkeitslehre," Zweite Ausgabe, Jena, 1913.

[4] Pearson, K., "On the Influence of Past Experience on Future Expectation," *Phil. Mag.*, March, 1907, pp. 365–378.

[5] Harris, J. A., "A Simple Test of the Goodness of Fit of Mendelian Ratios," Amer. Nat., Vol. XLVI, pp. 741–745, 1912. *Cf.* also Pearson, K., and Heron, D., "On Theories of Association," *Biometrika*, Vol. IX, pp. 159–315, 1913.

tical Mendelian work), and (2) the fact that the test takes into account only the *magnitude* of the error and not its *direction* (*i. e.*, whether in excess or defect) in any particular case. (3) It gives a result not particularly well adapted to the actual needs of Mendelian research. The χ^2 test gives a measure of the goodness of fit of the *whole* distribution, and *only* that. Now besides being interested in that point the Mendelian worker quite as often wants to know, in addition, something about the probability that *particular classes* observed are significantly different from the expected. To that sort of knowledge the χ^2 test helps him not at all. It is an "all or none" sort of method.[6]

2. It has seemed to the writer that it would be useful to discuss methods of determining the *true* probable error of each class frequency in Mendelian distributions as a supplement to the χ^2 test, and for use in cases where it is not applicable. The fundamental theorems have all been given by Pearson[7] in a very important paper, which is apparently almost entirely unknown to biologists. The purpose of the present paper is first to show the applicability of these theorems to the problem in hand, and second to point out some matters regarding the practical use of the method likely to be helpful to biologists with but little mathematical training who may attempt to use it.

3. In the paper referred to, Pearson, starting from Bayes' theorem, shows that the distribution of chances of an event occurring in a particular way in a second

[6] I have earlier pointed out other objections to the χ^2 test in Mendelian work, in particular its total failure to deal with cases where experiment yields a small frequency on classes where the expectation is zero, and need not further discuss them here. I have never thought it necessary to make any rejoinder to Pearson's characteristically bitter reply to my criticism, nor do I yet. The χ^2 test leads to this absurdity: if I perform a Mendelian experiment in which I get ten thousand million offspring agreeing *perfectly* with expectation save for *one* lone individual (perhaps a mutation, perhaps a mistake in the record, or what not) which is of a sort not expected, then Pearson and the χ^2 test agree that the probability is infinitely great that the ten thousand million offspring *do not* follow Mendelian law!

[7] Pearson, K., *loc. cit.*

sample from a population from which a first sample has produced a certain value is given, *not* by the ordinates of a normal curve of errors, as is commonly assumed in writings on the theory of probability, but by the successive terms of a simple hypergeometrical series. In an earlier paper the same author[8] had solved the problem of the momental properties of the hypergeometrical series. Combining the two results he was able to derive the necessary equations for the complete solution of the problem of probable errors in sampling. We may proceed at once to the exposition of these results, referring the reader for the proofs to the papers of Pearson cited.

Let it be supposed that a first sample of $n = p + q$ be drawn from the population, p denoting the number of times the event dealt with occurs in the n trials, and q the number of times it fails.

Write

$$p = \frac{p}{n}, \quad \bar{q} = \frac{q}{n},$$

whence of course

$$\bar{p} + \bar{q} = 1.$$

We then have for the chief constants of the error distribution for a *second sample,* of magnitude m, drawn from the same population the following values:

$$\text{Mean}^9 = m\bar{p} + \frac{m}{n+2}(\bar{q} - \bar{p}), \tag{i}$$

$$\text{Mode} = \text{the } \textit{integral} \text{ portion of } mp + p, \tag{ii}$$

$$\text{Standard Deviation} = \left\{ m\left(\bar{p} + \frac{\bar{q} - \bar{p}}{n+2}\right) \times \left(q - \frac{\bar{q} - \bar{p}}{n+2}\right)\left(1 + \frac{m-1}{n+3}\right)\right\}^{\frac{1}{2}}. \tag{iii}$$

These values are entirely general, and independent of the values of n, m, p and q. Under certain circumstances,

[8] Pearson, K., "On Certain Properties of the Hypergeometrical Series, and on the Fitting of such Series to Observation Polygons on the Theory of Chance," *Phil. Mag.*, Feb., 1899, pp. 236–246.

[9] From origin at the lower range end, or $r = 0$.

as when n is very large as compared with m, and neither p nor q are very small, (i) and (iii) are obviously capable of being put in much simpler form and still giving a sufficiently close approximation to the true result. For Mendelian work, however, where frequently neither of these conditions are even approximately realized, it will in general be better to use the full expression as given above.

The ordinates of the error distribution (the chances of different occurrences) are given by the successive terms of the hypergeometrical series

$$C_r = C_0 \left\{ 1 + \frac{m}{1} \cdot \frac{p+1}{q+m} + \frac{m(m-1)}{2} \cdot \frac{(p+1)(p+2)}{(q+m)(q+m-1)} + \frac{m(m-1)(m-2)}{\lfloor 3} \cdot \frac{(p+1)(p+2)(p+3)}{(q+m)(q+m-1)(q+m-2)} + \frac{m(m-1)(m-2)(m-3)}{\lfloor 4} \times \frac{(p+1)(p+2)(p+3)(p+4)}{(q+m)(q+m-1)(q+m-2)(q+m-3)} + \text{etc.} \right\}, \tag{iv}$$

where

$$C_0 = \frac{\Gamma(q+m+1)\Gamma(n+2)}{\Gamma(q+1)\Gamma(n+m+2)}. \tag{v}$$

As we shall presently see, the calculation of the terms in (iv) becomes a tedious and laborious matter when the number needed is at all considerable. Under such circumstances, and when in addition m and n are even moderately large, equation (iv) may be greatly simplified, without significant loss of accuracy, by the use of Sterling's theorem (to the bracket) or by Forsyth's approximation for such of the factorials as are not included in the range of the Pearson[10] tables. Thus we have, by Sterling's theorem, remembering that r denotes any term in the series, and writing $s = m - r$,

[10] "Tables for Statisticians and Biometricians," edited by Karl Pearson, Cambridge, 1914.

$$C_r = C_0 \frac{\lfloor p+r}{\lfloor p \lfloor r} \left\{ \frac{m^{m+\frac{1}{2}}(q+s)^{q+s+\frac{1}{2}}}{(q+m)^{q+m+\frac{1}{2}} s^{s+\frac{1}{2}}} \right\}. \qquad \text{(vi)}$$

Using Forsyth's approximation, which is extremely accurate, one gets

$$C_r = C_0 \frac{\lfloor p+r}{\lfloor p \lfloor r} \left\{ \frac{(m^2+m+\frac{1}{6})^{m+\frac{1}{2}}\{(q+s)^2+(q+s)+\frac{1}{6}\}^{q+s+\frac{1}{2}}}{(s^2+s+\frac{1}{6})^{s+\frac{1}{2}}\{(q+m)^2+(q+m)+\frac{1}{6}\}^{q+m+\frac{1}{2}}} \right\}^{\frac{1}{2}}. \qquad \text{(vii)}$$

The gamma terms in (v) will, of course, be calculated by some one of the well-known approximations (*e. g.*, Sterling's, Pearson's, Forsyth's) or by interpolation from a table of factorials (Pearl[11]).

4. The proposal which I wish to make for the expression of a Mendelian result is that *the expectation be expressed as the quartile limits for each class frequency in a second sample of the same size as the observed sample.* In using such an expression it must be clearly understood that it does *not* measure the goodness of fit of the distribution *as a whole,* because it takes no account of correlations in errors. What it does give, in supplement to the χ^2 test, is the limits of probability of each class frequency, *taken by itself.*

The ordinary expression for a probable error (*e. g.*, P. E. mean $= \pm .67449(\sigma/\sqrt{n})$) gives the quartile limits (*i. e.*, the limits within which one half the frequency occurs) on the assumption that the distribution is Gaussian, since in such a distribution of unit area the quartile limits are .6744898 . . . times the standard deviation on either side of the mean. But in our persent work we are making no assumption that the error distribution is Gaussian. Consequently we must determine the quartiles directly from the distribution. In cases where the number of terms is not too great the ordinates may be calculated from (iv) or (vi) and summed to find the quartile. In many cases, however, this would be practically too tedious

[11] Pearl, R., "Interpolation as a Means of Approximation to the Gamma Function for High Values of *n*," *Science,* N. S., Vol. XLI, pp. 506–507, 1915; "On the Degree of Exactness of the Gamma Function Necessary in Curve Fitting," *Ibid.,* Vol. XLII, pp. 833–834, 1915.

an operation, and we may resort to an approximate method. The simplest one is to take $.67449\sigma$ on either side of the median, which is approximately determined by remembering that the median lies between the mean and the mode and approximately twice as far from the mode as from the mean. The criterion of whether this method of fixing the quartile limits may be safely applied will be found in the value of the skewness, Sk. In practical work this approximate method will give sufficiently accurate results unless the skewness is very large, say > 0.6.

We have by definition

$$Sk = \frac{d}{\sigma}. \qquad \text{(viii)}$$

Hence having calculated the values of mean, mode and σ by (i), (ii) and (iii) we can readily obtain (viii), since $d =$ mean $-$ mode.

We may now pass to the consideration of some numerical examples, by means of which certain facts can be better brought out than by further theoretical discussion.

5. As a first and simple example we may take some data, recently published by F. L. Platt,[12] on the results of mating Blue Andalusian fowls. On account of the frequency with which the Blue Andalusian case is cited as a paradigm in Mendelism, coupled with the great dearth in the literature of exact statistics of actual matings of this breed of poultry, it seems especially worth while to discuss these statistics furnished by Mr. Platt, on the authority of Mr. W. J. Coates, a breeder of Andalusians.

Table I gives the data, and in the last line, the Mendelian expectation expressed in the form suggested in this paper.

The occurrence of the "dark reds," which Mr. Coates informs us had a pattern like a Red Game, is a phenomenon not mentioned in textbook accounts of Mendelian inheritance in the Blue Andalusian. In the present con-

[12] Platt, F. L., "Western Notes and Comment," *Reliable Poultry Journal*, Vol. XXIII, p. 665, 1916.

TABLE I

SHOWING THE RESULT OF MATING BLUE × BLUE IN THE BLUE ANDALUSIAN BREED OF POULTRY (COATES' DATA)

Mating	Offspring			
	White	Blue	Black	Dark Red
A	4	10	3	1
B	4	5	2	0
C	3	3	0	3
D	0	12	1	0
E	3	3	1	0
Total observed	14	33	7	4
Total observed in categories (1) white, (2) blue, (3) pigmented not blue	14	33	11	
If the true ratio is 1 white : 2 blue : 1 pigmented not blue it is an even chance, considering each class by itself, that the frequencies in a sample of this size will fall between	*11.5 and 17.8*	*25.8 and 32.9*	*11.5 and 17.8*	

nection, however, we can not pursue that point, but will group together, as in the penultimate line of the table, the blacks and reds as "pigmented, not blue," and assume that the three classes should occur in a 1:2:1 ratio. Do the actual results bear out this assumption, having regard to the errors of sampling?

Examining the last two lines of the table, it is clear that each observed class, taken by itself, is by no means an impossible approximation to what would be demanded by a 1:2:1 ratio. The blues and the "pigmented not blues" fall outside the range for which the probability is $\frac{1}{2}$ but only slightly outside. It would be practically an even bet, if Blue Andalusians really follow a law of 1:2:1 segregation when bred together, that any particular sample of 58 offspring would show in each particular class as great a deviation as the present sample.[13]

Now we may consider in detail the mode of calculating the figures in the last line of Table I.

[13] Always on the assumption, of course, that it is legitimate to lump the blacks and reds together. There is room for scepticism on that point, but we are here only concerned with the case as an illustration of method.

We have, by hypothesis, and from the statistics

$$m = n = 58.$$

A distribution of 58 in a 1:2:1 ratio is 14.5:29:14.5. Assume a first sample of 58 to show exactly this distribution 14.5:29:14.5, what will be the mean frequency of one of the end classes, say white, expected in a second sample of 58?

We have

$$\bar{p} = \frac{14.5}{58} = .25,$$

$$\bar{q} = .75,$$

whence, by (i),

$$\text{Mean} = 14.9833,$$

and, by (ii),

$$\text{Mode} = 14,$$
$$d = .9833.$$

By the approximate method we get

$$\text{Median} = 14.656 \text{ approximately.}$$

The standard deviation from (iii), is

$$\sigma = 4.6364,$$

and, by (viii),

$$Sk. = .2121.$$

Actual tests with curves of a degree of skewness no greater than this show that the approximate method gives the quartile limits with sufficient accuracy for practical purposes. We have for the approximate quartile limits, $.67449 \times 4.6364 = 3.1272$. This value, added to and subtracted from the median 14.656, gives the results set down in the last line of Table I.

Exactly the same procedure, with different numbers, is followed in the case of the blue column.

6. Let us now consider a more completely worked out illustration. Some time ago Mr. Alexander Weinstein, of Columbia University, consulted the writer in regard to a

problem which had arisen in connection with his Mendelian breeding experiments. A certain type of mating gave the following class frequencies:

$$6363 + 579 + 3638 + 1208 + 128 + \overset{(x)}{115} + 350 + 6 = 12387 \quad \ldots \quad (a).$$

Another group of matings gave a total of 9,017 offspring, of which 30 fell in the x class, this being the only class in regard to which a comparison is to be made. On certain theoretical grounds the percentage frequency in this x class in the second sample would be expected to be 0.582 times the percentage frequency of this same class in the first sample. The question is whether the actually observed frequency of 30 in this second sample is such as could reasonably be expected to occur if the theoretical assumption actually were the fact.

It will be seen at once that, owing to the very small absolute frequency of this x class in both samples, ordinary probable error methods will be of no avail.

Approaching the problem by the method here proposed, we have, as basic values for the computations,

$$n = 12387$$
$$m = 9017$$
$$p = 115,\ q = 12272$$
$$p = p/n = .009284$$
$$q = q/n = .990716.$$

Whence we have for the mean in the second sample of 9017 by (i)

$$\text{Mean} = 84.428137$$

and by (iii)

$$\sigma = 12.020652.$$

By (ii) the $mode = 83$, whence $d = 1.428137$

and

$$sk = \frac{1.428137}{12.020652} = 0.118807.$$

Working directly from the moments of the hypergeometrical series and, in effect, replacing that series with a true curve, we find

$$\begin{aligned} \text{Mode} &= 83.222141, \\ d &= 1.205996, \text{ and} \\ Sk &= 0.100327 \pm .008696. \end{aligned}$$

TABLE II

SHOWING THE SUCCESSIVE ORDINATES OF THE HYPERGEOMETRICAL SERIES FOR THE SECOND SAMPLE

r	C_r	*Sum*	r	C_r	*Sum*	r	C_r	*Sum*
35	.000000	.000000	73	.022620	.182838	111	.003215	.983125
36	.000001	.000001	74	.024355	.207193	112	.002740	.985865
37	.000001	.000002	75	.025996	.233189	113	.002325	.988190
38	.000002	.000004	76	.027539	.260728	114	.001964	.990154
39	.000003	.000007	77	.028943	.289671	115	.001651	.991805
40	.000005	.000012	78	.030183	.319854	116	.001382	.993187
41	.000008	.000020	79	.031236	.351090	117	.001152	.994339
42	.000013	.000033	80	.032085	.383175	118	.000957	.995296
43	.000020	.000053	81	.032715	.415890	119	.000791	.996087
44	.000031	.000084	82	.033116	.449006	120	.000651	.996738
45	.000046	.000130	83	.033285	.482291	121	.000533	.997271
46	.000068	.000198	84	.033220	.515511	122	.000436	.997707
47	.000099	.000297	85	.032929	.548440	123	.000354	.998061
48	.000142	.000439	86	.032419	.580859	124	.000287	.998348
49	.000200	.000639	87	.031706	.612565	125	.000231	.998579
50	.000279	.000918	88	.030805	.643370	126	.000186	.998765
51	.000383	.001301	89	.029738	.673108	127	.000149	.998914
52	.000520	.001821	90	.028526	.701634	128	.000119	.999033
53	.000696	.002517	91	.027193	.728827	129	.000094	.999127
54	.000919	.003436	92	.025763	.754590	130	.000075	.999202
55	.001199	.004635	93	.024262	.778852	131	.000059	.999261
56	.001545	.006180	94	.022711	.801563	132	.000046	.999307
57	.001967	.008147	95	.021136	.822699	133	.000036	.999343
58	.002476	.010623	96	.019556	.842255	134	.000028	.999371
59	.003082	.013705	97	.017991	.860246	135	.000022	.999393
60	.003793	.017498	98	.016459	.876705	136	.000017	.999410
61	.004617	.022115	99	.014974	.891679	137	.000013	.999423
62	.005561	.027676	100	.013550	.905229	138	.000010	.999433
63	.006629	.034305	101	.012195	.917424	139	.000008	.999441
64	.007821	.042126	102	.010917	.928341	140	.000006	.999447
65	.009135	.051261	103	.009722	.938063	141	.000005	.999452
66	.010569	.061830	104	.008614	.946677	142	.000004	.999456
67	.012109	.073939	105	.007593	.954270	143	.000003	.999459
68	.013743	.087682	106	.006660	.960930	144	.000002	.999461
69	.015455	.103137	107	.005813	.966743	145	.000001	.999462
70	.017223	.120360	108	.005049	.971792	146	.000001	.999463
71	.019025	.139385	109	.004364	.976156	147	.000001	.999464
72	.020833	.160218	110	.003754	.979910			

The two sets of values are evidently sufficiently near together to be used interchangeably for most practical

purposes, a sort of result which is familiar to any one who has had any considerable experience with the method of moments.

To return now to the series we find, using 10-place logarithms in the intermediate computations and the Forsyth approximation,

$$\log C_0 = 72.3493814 - 100.$$

We have now to calculate the successive terms of the series. If this were done for the whole range it would involve a literally colossal amount of labor. Fortunately this is not necessary. We need only take that part of the range which includes appreciable frequencies. By a few trials we find that this part of the range begins with $r = 36$. In Table II are given the frequencies for the several terms in the series between $r = 36$ and $r = 147$ inclusive, the total area being taken as unity. To reduce these frequencies to the actual numbers for the second sample we have only to multiply in every case by 9017. We have calculated C_{36} by (vii) and used the Forsyth C_0.

From this table we easily deduce

$$\begin{aligned} \text{Median} &= 83.5331, \\ \text{Lower quartile} &= 75.6104 \\ \text{Upper quartile} &= 91.8218. \end{aligned} \qquad \text{(ix)}$$

Now, remembering that if the same law holds for the second Mendelian distribution as for the first we should expect the x class in that distribution to be 0.582 times the value of the same class in the first distribution, we have

Expected mean value of x class in second distribution = 49.14

Expected modal value of x class in second distribution = 48

Expected lower quartile value in second distribution = 44.01

Expected upper quartile value in second distribution = 53.44

The actual experimental value obtained was 30, which is far below the lower quartile. From Table II we find, remembering again that on *a priori* grounds the experimental frequencies are reduced by the factor 0.582, that if the two distributions were really samples of the same population, obeying the same Mendelian laws, it would be expected that the x class would show a frequency as low as or lower than 30, only 18 times in 10,000 trials of samples of 9017. Or, in other words, the odds against so low a value as 30 are about 556 to 1. These are about the same odds as those associated with the occurrence of a deviation 4.63 times the probable error (cf. Pearl and Miner[14]).

We may, therefore, conclude with great certainty that the value of 30 is significantly *smaller* than would be expected to occur in the x class on the basis of chance (deviation due to random sampling) if the two distributions were really samples of the same population.

Let us now go back and approach the problem *de novo* by the approximate method suggested in section 4. We have

$$d = \text{mean} - \text{mode} = 1.4281,$$

$$\frac{1.4281}{3} = .4760,$$

$$\begin{aligned}
84.4281 - \ .4760 &= 83.9521 = \text{median (approx.)},\\
12.0207 \times \ .67449 &= \ 8.1078,\\
83.9521 - 8.1078 &= 75.8443 = \text{Lower quartile},\\
83.9521 + 8.1078 &= 92.0599 = \text{Upper quartile}.
\end{aligned}$$

Comparing these values, in the obtaining of which all the tremendously tedious and time-consuming arithmetic involved in calculating Table II was avoided, with those shown in (ix) makes it quite evident that for all practical statistical purposes the approximate method would have given sufficiently accurate results.

[14] Pearl, R., and Miner, J. R., "A Table for Estimating the Probable Significance of Statistical Constants," Me. Agr. Expt. Stat. Ann. Rept. for 1914, pp. 85–88.

SUMMARY

In this paper is presented a method of calculating and expressing the errors, due to random sampling, of a Mendelian class frequency. The method consists essentially in expressing each *expected* Mendelian frequency as the probable quartile limits for that class frequency in a supposed second sample of the same size as the observed sample drawn from the same population. These quartile limits are determined from the ordinates of a hypergeometrical series. Various simplifications of method are suggested and illustrated. The method is suggested as a supplement to, not as a substitute for, the χ^2 test for the goodness of fit in Mendelian distributions.

OBSERVATIONS ON THE ECOLOGY OF THE PROTOZOA

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A great deal of excellent work has been done in census taking among the Protozoa, and numerous catalogues of species with descriptions and accompanying plates have been issued for various states or portions thereof. This present communication, however, is a slight contribution toward census taking of a different sort, in that it aims to set forth some facts regarding the different types of Protozoan habitats and the species usually associated with each. It is well known that certain common forms, used as type species in the laboratory (such as *Amœba proteus* and *Paramœcium caudatum*), can be found only in certain fairly definite environments. The ecology of such forms is well known, but the bionomics of the majority of the Protozoa is still virtually a *res ignota*. The study of environmental units and Protozoan communities will contribute to a more intimate knowledge of these elusive forms.

Although little is definitely known concerning the ecology of the Protozoa, yet I think that we are in position to say that Protozoan inhabitants vary with their varying environments. A record of the inhabitants of a marsh pool will not include the same species normally found in clear running streams, nor will cold waters yield the same forms as warm. But we can go still farther, I think, and say that the various portions of any given environmental unit, even though they differ ever so slightly from one another, will each have its own characteristic group of organisms. The factors which are accountable for the variation in species or numbers of individuals of any species in such instances may be hardly discoverable, but they are none the less potent. Thus the entrance point of a tiny thread

of clear spring water in the midst of an impure pool may furnish an abundance of *Monas* and *Cercomonas,* and because of the presence of these, also the larger carnivorous forms such as *Dileptus* or *Onychodromus.* Or the growth of myriads of bacteria at another point may induce the increase in the numbers of *Vorticellæ, Amœbæ,* or *Holophryæ.* A complete study of the multitudinous minute variations in the environments of Protozoa, and their complex relationships with the associated species is still far away. But I believe that we are now ready to undertake a preliminary survey of a number of typical environments and to ascertain what genera and species may be normally expected to occur in each.

Of the many factors responsible for the localization of species, the following are perhaps first in importance: light, character of food, temperature of water, chemical content of water, presence or absence of enemies.

Light exerts a powerful influence in determining the distribution of species within a given body of water. Thus one will find certain species in sunlit areas and certain others in shaded ones. Not all of the Protozoa respond to ordinary light stimuli, as has been shown experimentally by Jennings.[1] He has found that comparatively few of the ciliate infusoria react to light stimuli. The flagellates, on the other hand, show a definite reaction, congregating in that region where the light is ordinarily strongest. The reactions of *Euglena viridis* and *Cryptomonas ovata* are the forms which have been most carefully studied in this regard.

Jennings[1] further showed that *Paramœcia* collect in those regions of the water which contain a trace of any weak acid, and this I have noted to be true of certain other ciliates. This may be a dominant influence in localizing forms in pools and streams in marshes where organic acids are usually present. The presence of supernormal amounts of oxygen, carbon dioxide, calcium carbonate, iron, and other dissolved substances may also affect the distribution of species.

[1] "Contributions to the Study of the Behavior of Lower Organisms," H. S. Jennings, Washington, 1904.

Not only may the various stimuli arising from the different elements of the environment assort species into communities, but the effects of these stimuli may not always be the same. A given stimulus may at one time produce a certain type of reaction, and at another time a somewhat different type. This fact, also, experimentally verified by Jennings, led him to suppose that something analogous to, if not identical with true varying "physiological states" obtains in the Protozoan body. If this be so then the problem of Protozoan ecology is still further complicated, as far as any assignment of species to definite fixed local environments is concerned.

For collecting Protozoa and water samples the writer has found the following useful: a small silk plankton net (with draw string bottom) about six inches in diameter and ten inches deep; a glass or metal pipette, fully a foot in length, operated by a compression bulb at one extremity, for sucking samples of light sediments from the bottoms of pools and streams; several small glass dipping tubes; a large table spoon; a thermometer, and a plentiful supply of variously sized water tight jars with screw tops. With such an outfit as this the material described in the following paper was secured. The samples were examined immediately after being brought into the laboratory, in order that the proportions of species might be accurately recorded.

From each sample ten slides were searched. The significance of the terms few, numerous, rare, etc., is as follows:

Over ten individuals of one species per slide .....abundant
Average of 5–10 .....numerous
Average of 2–5 .....several
Average of .5–2 .....few
Average of less than .5 .....rare

The samples were taken from the uppermost layer of silt on the bottoms of pools, streams, etc., the utmost care being taken not to disturb the sediment below or to roil the water. Upwards of fifty samples were secured representing five distinct environmental types. These five types described are:

No. 1. Characteristic marsh pools.

No. 2. Clear cold lakes and streams with no plant life.

No. 3. Clear springs and streams with plants abundant.

No. 4. Small, clear pools containing organic sediment in decomposition and fed by pure, cold rills.

No. 5. Ditches and pools choked with algæ, water warm.

All of the material was taken during the months of late spring, summer, and early fall, while the aquatic vegetation, and the semiaquatics about the water margins, were in vigorous growth.

ENVIRONMENTAL TYPE NO. I: MARSH POOLS

Characteristic frog-inhabited marsh pools, containing much decaying vegetable matter, supporting rank growths of typical marsh plants about the margins, covered with lily pads and filled with *Ceratophyllum, Myriophyllum, Sagittaria,* duckweed, etc. Water warm, and emitting characteristic swampy odor.

Predominant forms:

Amœba limax, several
Amœba proteus, several
Amœba radiosa, several
Arcella vulgaris, numerous
Carchesium polypinum, numerous
Codonocladium umbellatum, numerous
Coleps hirtus, abundant
Coleps sp., abundant
Difflugia acuminata, numerous
Difflugia corona, numerous
Difflugia globulosa, numerous
Euglypha alveolata, numerous
Oikomonas sp., numerous
Peridinium cinctum, abundant
Stylonychia mytilus, numerous
Stylonychia pustulata, numerous
Stentor polymorphus, numerous
Synura uvella, numerous
Trinema acinus, numerous
Vorticella microstoma, numerous
Vorticella nutans, numerous

Other forms present in less numbers (few or rare):

Actinophrys sol
Arcella dentata
Arcella discoides
Anisonema obliqua
Astasia sp.
Biomyxa vagans
Blepharisma lateritia
Centropyxis aculeata
Colpoda sp.
Cothurnia maratima
Dactylosphærium radiosum
Dinomonas vorax
Euplotes carinata
Euplotes charon
Halteria grandinella
Heterophrys myriapoda
Holophrya sp.
Lacrymaria olor
Lionotus wrzesmosky
Lionotus sp.
Loxophyllum sp.
Paramœcium bursaria
Pleuronema sp.
Prorodon griseus

Stentor cæruleus
Trichodina pediculus (on *Hydra fusca*)
Urocentrum turbo
Volvox globator
Vorticella sp.

Blepharisma lateritia (Fig. 1) is usually described as showing pink or even reddish hues, and occurring rarely colorless. My experience has been to find the greater number of individuals colorless and a very few showing even a faint trace of pink. In reproducing the colors of Protozoa in plates there seems to be a tendency to represent them more vividly than they occur in nature. It is interesting to place a small chart of spectrum colors on the table near the base of the microscope and compare them with the hues of those species of Protozoa usually represented as brightly colored. It has been my experience to find that the (depicted) decided colors of some of the Protozoa show themselves to be only the faintest tints.

Synura uvella (Fig. 2) is not entirely colonial in its habit. I have found it sometimes singly, and often in pairs. The normal number of individuals in a colony is from 10 to 20. As many as 35 in one group have been recorded. The flagella are invisible unless iodine or some good stain has been used.

The *Coleps* sp. (Fig. 3) which I found so frequently in this one group of samples I have never found since. It was appreciably smaller than *Coleps hirtus* (Fig. 4), being about 30 microns in length, whereas *hirtus* is 50. *Hirtus* is a species exhibiting a notable constancy in dimensions, and this I found true also of all the numerous individuals of *Coleps* sp. which I measured. No transitions in size from one to the other could be found. *Coleps* sp. was not the result of fission on the part of *hirtus*, for in all the material my search revealed not a single individual undergoing division. In appearance and activities the one form was the exact counterpart of the other, with a solitary exception: the movements of *Coleps* sp. were at all times much more rapid than those of *hirtus*.

Lacrymaria olor (Fig. 5) described as a very variable species, is variable in *size* almost entirely. Its *form* is quite constant, and offers a virtually certain criterion for identification.

PLATE I

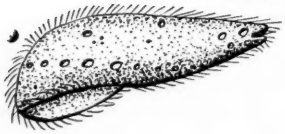


Fig.I Blepharisma lateritia

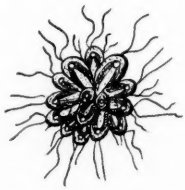


Fig.2 Synura uvella

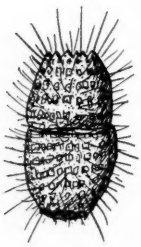


Fig.4 Coleps hirtus

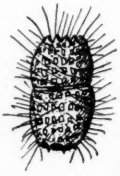


Fig.3 Coleps sp.

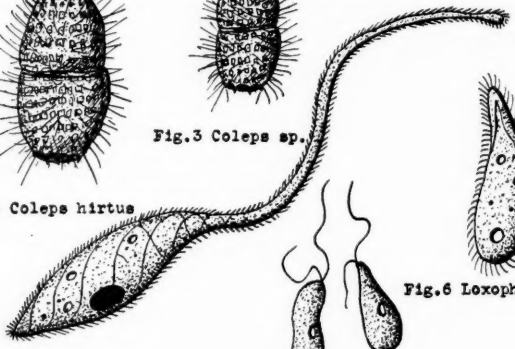


Fig.5 Lacrymaria olor

Fig.6 Loxophyllum sp.

Fig.7 Astasia contorta

Fig.8 Urocentrum turbo

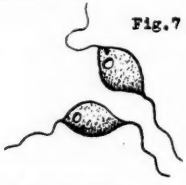


Fig.9 Cercomonas crassicauda

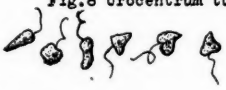
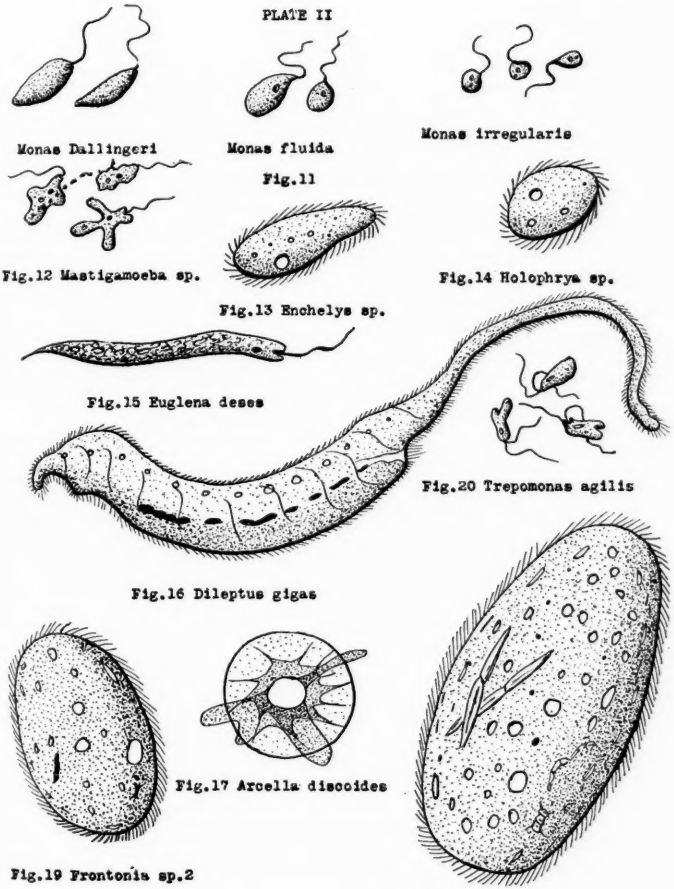


Fig.10 Cercomonas termo, normal and abnormal forms

PLATE II


Monas Dallingeri
Monas fluida
Monas irregularis
Fig.11
Fig.12 Mastigamoeba sp.
Fig.13 Enchelys sp.
Fig.14 Holophrya sp.
Fig.15 Euglena deses
Fig.20 Trepomonas agilis
Fig.16 Dileptus gigas
Fig.17 Arcella discoides
Fig.19 Frontonia sp.2
Fig.18 Frontonia sp.1

The body of *Loxophyllum* (Fig. 6) is surprisingly elastic. For this reason the creature undergoes rapid variations in length. Even within the confinement of a viscous gelatin solution which checks the motions of all of the other ciliates its strength is such as to enable it to perform various evolutions over the slide. In staining it frequently dies in a contorted and contracted condition. Before staining, it should be narcotized with a weak ethyl alcohol or killed with .01 per cent. osmic acid.

In *Astasia* (Fig. 7) the smaller secondary flagellum is sometimes either unusually filamentous, and therefore invisible, or lacking altogether. In many individuals no amount of manipulation of light directions and intensities or stains will reveal the smaller flagellum.

Urocentrum turbo (Fig. 8) can always be recognized by the frenzied and jerky rotation which accompanies its extremely rapid movement through the water. It usually comes to rest either close beside or buried among masses of algæ or other convenient material. In this resting condition it is liable to be overlooked. At such times its only evidence of activity is a scarcely perceptible twitching. This hiding in algal masses has all the appearance of being a deliberate attempt at concealment. Suddenly without warning its crazied rotation through the water is begun again. When swimming with retarded velocity through a gelatin solution, functional anterior and posterior portions of the body are recognizable.

ENVIRONMENTAL TYPE NO. II: CLEAR COLD WATERS LACKING PLANT GROWTHS

Sediment, composed of quartz and shale sands free from organic silt and supporting no plant life save a few Diatoms, in clear, pure, cold lakes and streams; temperature of water, cir. 56° F.

Predominant forms:

Astasia sp., abundant
Holophrya sp., numerous
Holostichia vernalis, numerous
Nostolenus orbicularis, several
Nostolenus sp., several

Other forms present in less numbers (few or rare):

Cercomonas termo
Cercomonas crassicauda
Chlamydomonas sp.
Colpidium sp.
Euplotes sp.
Lionotus sp.
Paramœcium bursaria
Stylonychia mytilus

The nucleus of *Cercomonas crassicauda* (Fig 9) is located near the functional anterior end of the body, and is not easily seen. The anterior flagellum is likewise difficult to distinguish, even upon staining, being exceedingly filamentous. It seems to have little to do with locomotion, and I believe that it is used principally as a sort of antenna. The propulsion of the creature is apparently accomplished by the lashings of the stout posterior flagellum, no matter which end of the organism is directed forward.

Cercomonas termo (Fig. 10), from 5 to 15 microns, is extremely variable in shape in the adult form. Its commonest and therefore characteristic appearance is roughly heart shaped, the flagellum arising from the broader extremity of the body. The young are not so variable, but although constant in general form, confusingly resemble the monads, in particular *Monas irregularis*. Their minuteness (.5 to 2 microns) makes them difficult to identify. They should be stained with a very weak solution of iodine or acetic methyl green, since the stronger stains, used for the larger Protozoa, cause them to lose their characteristic outline and to disintegrate in a short time.

Environmental Type No. III: Clear Flowing Water with Abundant Plant Life

Clear springs and streams supporting vigorous growths of such algæ as *Spirogyra, Drapernaldia,* etc., and water cresses; bottoms covered with Diatoms (often chiefly *Meridion circulare*), Desmids, and *Oscillatoria*. Temperature of water, cir. 66° F.

Predominant forms:

Amœba proteus, abundant
Amœba proteus, flagellospores (?), abundant
Chilodon cucullus, abundant
Chilomonas paramœcium, abundant
Colpidium sp., several
Colpoda inflata, several
Difflugia constricta, numerous

Difflugia globulosa, numerous
Difflugia lobostoma, numerous
Holophrya sp., several
Holostichia vernalis, several
Monas fluida, abundant
Monas irregularis, abundant
Monas Dallingeri (?), abundant
Nostolenus orbicularis, numerous
Oxytrichia pellionella, abundant
Prorodon teres, numerous

Other forms present in less numbers (few or rare):

Arcella artocrea
Aspidisca costata
Astasia contorta
Cercomonas crassicauda
Chilodon sp.
Chlamydomonas sp.
Colpidium striatum
Dileptus gigas
Enchelys sp.
Epiclintes radiosa
Euglena viridis
Euglena minima
Frontonia sp. 2
Holophrya sp.
Lionotus wrzesmosky
Mastigamœba sp.
Onychodromus grandis
Oxytrichia bifaria
Phacus sp.
Paramœcium caudatum
Stylonychia mytilus
Stylonychia pustulata

The identification of the *Monas* species (Fig. 11) is very difficult. Members of this genus resemble some of the members of the genus *Bodo*. Because of the hyaline character of the body its outline is not easy to distinguish. I have found that the most successful method of treatment before identification is attempted, is staining with a strong aqueous solution of iodine and potassium iodide. This both kills and stains. Examination must be made almost immediately since the organisms begin to lose their characteristic form in a short time.

Within the genus *Holophrya* have been provisionally placed a number of forms very closely allied in general characters. For a considerable number of these a separation into species has not yet been made.

The pseudopodia of the Mastigamœbæ (Fig. 12) do not invariably disappear with the appearance of the flagellum. They often remain, though much diminished in size, and even exhibit appreciable movement. Such movement, however, does not appear to aid in locomotion.

Enchelys (Fig. 13) is very like *Holophrya* (Fig. 14) and only under the most favorable conditions of staining and lighting can the side opening buccal orifice be definitely located. The proboscis-like projection at the anterior extremity of the body is subject to considerable modification, and can not be relied upon as a distinguish-

ing feature, but the position of the buccal cavity is virtually constant.

Euglena deses (Fig. 15) is recognizable by its enormously elongated body, for the propulsion of which its small flagellum seems inadequate. It frequently assumes an almost amœboid form, and not infrequently contracts to such an extent as to become nearly globular. Being variable in size, its form, when swimming by means of its flagellum, offers the readiest means of identification.

One particular locality, given especial notice, deserves detailed mention. This was a spring pool in a boggy upland meadow, almost hidden among large tufted clumps of luxuriant sedge-like grasses. The depth of the pool was about four inches, and its area about six square feet. Although exposed to the rays of the sun during the greater part of the day, yet the temperature of the water was kept low by a constant trickle of cold water from seepage springs in the glacial clays which underlay several acres of the region. The iron content of the water was unusually high, though the pool was crystal clear. Of higher aquatic plants there were none; Desmids were numerous; Diatoms were sufficient in numbers to form a brown film over the entire bottom. The pool contained seven large black-nosed dace, and other adjacent pools harbored several more. Snails, *Limnea* and *Physa* crawled everywhere. With the exception of half a dozen large *Hydrophilidæ* there were no aquatic insects, and but very few *Entomostraca.*

The predominant forms were *Monas fluida* and *irregularis,* occurring not alone in the bottom film but everywhere throughout the water and in great numbers. One species of *Holophrya* and *Cercomonas crassicauda* (but few individuals of each) completed the census of the forms.

Environmental Type No. IV: Clear Small Pools with Abundant Decomposing Organic Sediment

Small clear pools in depressions in rocks containing decaying leaf material and a small quantity of such algæ as

Spirogyra, Zygnemea, Mougeotia, etc., Desmids and Diatoms abundant. Water kept pure and cool by influx from small rills or by seepage from adjacent stream. Temperature of water, cir. 60° F.

Predominant forms:

Chilodon cucullus, numerous
Chilomonas paramœcium, abundant
Coleps hirtus, numerous
Difflugia globulosa, numerous
Holophrya sp., numerous
Holostichia vernalis, numerous
Monas sp., abundant
Paramœcium bursaria, numerous
Trachelocerca olor, numerous
Vorticella nutans, several

Other forms present in less numbers (few or rare):

Amœba proteus
Anisonema obliqua
Arcella vulgaris
Aspidisca costata
Chilodon vorax
Chlamydomonas sp.
Colpoda cucullus
Cyphoderia ampulla
Dallasia frontinia
Difflugia acuminata
Difflugia pyriformis
Dileptus gigas
Dileptus monilatus
Euglena deses
Euglena viridis
Euglypha alveolata
Euplotes sp.
Frontonia sp.
Hyalosphenia papilio
Loxophyllum sp.
Nostolenus orbicularis
Oxytrichia bifaria
Oxytrichia pellionella
Paramœcium caudatum
Pleuronema sp.
Prorodon armatus
Stylonychia pustulata
Stylonychia putrina

Dileptus gigas (Fig. 16) is surely the king of beasts among the ciliate Protozoa. It is entirely carnivorous and its appetite is apparently insatiable. The prey is stung by the well developed trichocysts which *Dileptus* bears upon its long "neck" and if too large to be swept into the buccal cavity by the cilia is forced in by the writhings of the "neck." The creature varies greatly in size, but is normally about 450 microns in length. Individuals have been reported measuring 800 microns!

ENVIRONMENTAL TYPE NO. V: POOLS CHOKED WITH ALGÆ; WATER WARM

Ditches and pools choked with heavy, luxuriant masses of algæ (*Spirogyra, Ulothrix, Zygnemea,* etc.) and exposed to the sun during the entire length of the day. *Cyclops, Canthrocamptus, Gammarus, Daphnia, Cypris, Simocephalus,* etc., abundant. In lesser numbers: aquatic

insects (especially the *Hydrophilidæ*), *Limnea, Physa, Planorbis*, etc. Temperature of water, cir. 70° F.

Predominant forms:

Cercomonas termo, abundant
Chlamydomonas sp., numerous
Difflugia globulosa, numerous
Euglena viridis, very abundant
Monas fluida, very abundant
Monas irregularis, very abundant
Peridinium cinctum, numerous
Synura uvella, numerous
Trepomonas agilis, very abundant

Other forms present in less numbers (few or rare):

Actinophrys sol
Arcella discoides (?).
Arcella mitrata
Arcella vulgaris
Colpoda campyla
Cyphoderia ampulla
Frontonia sp.
Vorticella nutans

The species which I identified as *Arcella discoides* (Fig. 17) was much smaller than usual. Although there is considerable variation in size among the members of *Arcella* and particularly among *Arcella discoides* and *vulgaris*, yet I have never seen recorded individuals so small. The average diameter of twenty-five individuals from one sample was 70 microns.

The *Frontonia* species (Figs. 18 and 19) are very fully equipped with trichocysts. When irritated with acetic acid and then stained with iodine or methyl green they show beautifully. For use in the laboratory in the demonstration of the trichocysts of the ciliata they offer the best possible material. The species are not very common, however, and I know of no culture medium.

Trepomonas agilis (Fig. 20) varies greatly in size. Its apparent variation in shape can be explained, I think, by the fact that it swims sometimes with one aspect of the body presented to the observer and sometimes with another. The curious irregularity of its body would therefore allow it to show a number of different outlines. Often it swims in one position for a long period, and again it twirls rapidly about going through the whole gamut of its apparent changes of form in a few seconds.

In one typical roadside ditch overhung with grasses and weeds and literally filled to overflowing with rich masses of *Spirogyra* I found an almost pure culture of *Euglena*

viridis. The only other forms present were but very few *Colpoda campyla* and *Cyphoderia ampulla*.

Several samples were taken from leaves and grass frozen together, lying beneath three inches of solidly compacted snow in a small oak grove not far from a ravine through which flowed a perennial stream. This material was allowed to stand in its own snow in a cotton-tamponed jar until the snow had melted and the resulting water had attained room temperature, cir. 72° F.

The *Amœba proteus* were more abundant than I had ever seen them before except in artificial cultures. Not infrequently as many as ten individuals could be counted at once in the field of the 10 mm. objective. The other predominant forms were:

Holostichia vernalis, abundant.
Monas irregularis, abundant.
Opalina ranarum, several
Paramœcium caudatum, several

Other forms present in less numbers (few or rare):

Astasia lagenula
Oikomonas sp.
Paramœcium trichium
Platyrichotus opisthobolus

CATALOGUE OF SPECIES NOTED WITH MEASUREMENTS IN MICRONS

In each case *length* refers to the antero-posterior axis, except with globular or subglobular forms when it refers to the diameter of the body. All dimensions are given in microns (=1/1000 mm.).

It is interesting to recall, in connection with the sizes of these organisms, that the diameter of the average human hair is 100 microns.

Genus	Species	Length
Actinophrys	*sol*	60
Amœba	*limax*	50–60
Amœba	*proteus*	125 (variable)
Amœba	*radiosa*	50–100 (variable)
Anisonema	*obliqua*	
Arcella	*artocrea*	160
Arcella	*dentata*	95
Arcella	*discoides*	115
Arcella	*mitrata*	100–150
Arcella	*vulgaris*	55 (variable)
Aspidisca	*costata*	30

Astasia	*contorta*	30 (variable)
Astasia	*lagenula*	20
Astasia	sp.	75
Biomyxa	*vagans*	50 (variable)
Blepharisma	*lateritia*	170
Carchesium	*polypinum*	60 (bell only)
Centropyxis	*aculeata*	125–150
Cercomonas	*crassicauda*	25
Cercomonas	*termo*	5–15
Chilodon	*cucullus*	135
Chilodon	*vorax*	70
Chilodon	sp.	42
Chilomonas	*paramœcium*	40 (variable)
Chlamydomonas	sp.	17
Codonocladium	*umbellatum*	8–12
Coleps	*hirtus*	50
Coleps	sp.	30
Colpidium	*striatum*	45
Colpidium	sp.	34
Colpoda	*campyla*	70
Colpoda	*cucullus*	95
Colpoda	*inflata*	45
Colpoda	sp.	50
Cothurnia	*maratima*	85
Cyphoderia	*ampulla*	75
Dactylosphærium	*radiosum*	25 (variable, body only)
Dallasia	*frontinia*	150
Difflugia	*acuminata*	100–300
Difflugia	*constricta*	50–100
Difflugia	*corona*	150–250
Difflugia	*globulosa*	20–50
Difflugia	*lobostoma*	90–120
Difflugia	*pyriformis*	250–350
Dileptus	*gigas*	450 (variable)
Dileptus	*monilatus*	200
Dinomonas	*vorax*	18 (variable)
Enchelys	sp.	50
Epiclintes	*radiosa*	50
Euglena	*deses*	50–200
Euglena	*minima*	30 (variable)
Euglena	*viridis*	50 (variable)
Euglypha	*alveolata*	75
Euplotes	*carinata*	80
Euplotes	*charon*	90
Euplotes	sp.	35
Frontonia	sp. 1	300–450
Frontonia	sp. 2	100
Halteria	*grandinella*	25
Heterophrys	*myriapoda*	60
Holophrya	sp.	25–45

Holostichia	*vernalis*	50
Hyalosphenia	*papilio*	125
Lacrymaria	*olor*	
	extended	360
	contracted	90
Lionotus	*wrzesmosky*	225
Lionotus	sp.	30
Loxophyllum	sp.	50
Mastigamœba	sp. (*repetans?*)	20
Monas	*Dallingeri*	These vary between 1 and 5
Monas	*fluida*	
Monas	*irregularis*	
Nostolenus	*orbicularis*	35
Nostolenus	sp.	17
Oikomonas	sp.	10–20
Onychodromus	*grandis*	100–350
Opalina	*ranarum*	115
Oxytrichia	*bifaria*	100–280
Oxytrichia	*pellionella*	80–150
Paramœcium	*bursaria*	90–150
Paramœcium	*caudatum*	150–300
Paramœcium	*trichium*	85
Phacus	sp.	50
Platyrichotus	*opisthobolus*	50
Pleuronema	sp.	50
Prorodon	*armatus*	25
Prorodon	*griseus*	40
Prorodon	*teres*	150
Stentor	*cæruleus*	100–400
Stentor	*polymorphus*	100–300
Stylonychia	*mytilus*	80–150
Stylonychia	*pustulata*	80–150
Stylonychia	*putrina*	50 (variable)
Synura	*uvella*	
	individual	10
	colony	35
Trachelocerca	*olor*	70–130
Trepomonas	*agilis*	8–10
Trichodina	*pediculus*	230
Trinema	*acinus*	60
Urocentrum	*turbo*	45
Volvox	*globator*	
Vorticella	*microstoma*	45
Vorticella	*nutans*	70
Vorticella	sp.	60

THE RÔLE OF ISOLATION IN THE FORMATION OF A NARROWLY LOCALIZED RACE OF DEER-MICE (PEROMYSCUS).*

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No one who has critically examined large numbers of specimens, belonging to such a widely distributed and diversified genus as *Peromyscus*, can fail to be impressed with two facts. First, the differences upon which the so-called "subspecies" are based are real and obvious ones. But, secondly, the actual subspecies which are recognized and named are necessarily highly artificial groups. On the one hand, each subspecies intergrades with others to such an extent that the assignment of a given specimen to one or the other group is often quite arbitrary. And on the other hand, even these "subspecies" themselves are far from being elementary. They are composite groups, comprising, in many cases, a number—perhaps a great number—of distinguishable local types. The word *distinguishable* is here used in a qualified sense. It is likely that the distinctions would commonly be obvious just in proportion as the collections were made at points which were remote from one another.

Indeed, it has been said by one who has monographed this genus of mice[1] that "classification becomes . . . like dividing the spectrum and depends largely upon the standards set, for, theoretically at least, the possibilities of subdivision are unlimited (p. 17)."

None the less, it is generally believed that where well-marked physical or other barriers are interposed between two groups of individuals, this continuous intergradation

* Read before Ecological Society of America, San Diego meeting, August, 1916.

[1] Osgood, "Revision of the Mice of the American Genus, *Peromyscus*," North American Fauna, No. 28. Washington, 1909.

of racial characters may be largely interrupted. It is the object of this paper to discuss a case of this sort which I have had the opportunity of studying during the past year.

The subspecies *Peromyscus maniculatus rubidus,* according to Osgood,[2] who first described it, occupies a strip of varying width on the "coast of California and Oregon from San Francisco Bay to the mouth of the Columbia River." In discussing certain local variations shown by this subspecies throughout its range, the same writer states that "six specimens from the Outer Peninsula, near Samoa, Humboldt Bay, are decidedly paler than others from the neighboring redwoods. They evidently represent an incipient and very local subspecies, and well illustrate the plasticity of the group to which they belong." Osgood further remarks that "a careful study of this variation and the local conditions doubtless would prove instructive" (p. 66).

During the latter part of May, 1916, I trapped on two consecutive nights in the neighborhood from which Osgood obtained his six "aberrant" specimens of *rudibus.*[3] About one hundred live-traps were set on each occasion. Twenty-eight specimens were taken, of which twenty-one were later available for skinning and for careful measurement. These last were all in either mature or adolescent pelage, and were about evenly divided in respect to sex.

The distinctness of this race from the *rubidus* of the redwood forests on the mainland was evident from a casual inspection of the living mice. A more careful comparison of freshly killed specimens from the two localities, and later of their prepared skins, justifies the following generalizations. These impressions were formed independently by several other persons to whom I showed the specimens, and were confirmed by more careful ex-

[2] *Loc. cit.,* p. 65.

[3] The trapping was done between one and two miles northwest of the village of Samoa. Besides these *Peromyscus,* the only other animal caught was a single specimen of *Microtus.*

amination and measurement. (1) The Samoa lot, as a whole, were paler than the redwood lot; (2) the tails of the former were shorter, and (3) the ears were longer.

To consider first the coat color, the mean difference between the two series of skins is evident at a glance. Likewise, it is plain that the palest Samoa specimen is paler than the palest Eureka (redwood) specimen, and that the darkest among the former is paler than the darkest among the latter. It must be admitted, however, that the two series overlap rather broadly,[4] the darker skins of the Samoa stock being as dark as or darker than the paler ones of the Eureka stock.

An attempt to express the color of a mammal's pelage in terms of any set of "standard" colors is beset with great difficulties. Instead of a uniformly tinted, plane surface, we have to do with a mixture of variously colored hairs, further diversified by minute shadows and reflections. I have, nevertheless, endeavored, in a rough way, to "match" the colors of these two races with those of Ridgeway's "Color Standards and Color Nomenclature."[5] In the Samoa race, the general tone of the lateral regions of the body lies between the "tawny olive" and "Saccardo's umber," that of the dorsal darker stripe being not far from "sepia." In the Eureka mice, the lateral regions range from "Saccardo's umber" to "sepia," the dorsal stripe being of a depth somewhere between "sepia" and black. These comparisons will at least enable the reader to judge of the degree of difference between the two races.[6]

As regards the tail, it was plain without measurement that the average length of this member was greater in the

[4] I have at present for reference twenty-one skins of the Samoa lot and thirty skins of wild adults from the redwoods. Ten of the latter individuals were trapped and skinned at about the same time as the former, so that the factor of season may be disregarded.

[5] Washington, 1912. Published by the author.

[6] In my further studies of *Peromyscus* I plan to employ two revolving color-wheels, on one of which the skin itself will be rotated, on the other sectors of black, white and various primary colors. This apparatus is now being tested by Mr. H. H. Collins and myself.

Eureka than in the Samoa race, though here again the difference related to averages and did not hold for all individual cases.

A comparison of the mean figures for *absolute* tail length in two series of mice is not entirely justifiable, particularly if the two lots of individuals differ somewhat in mean body size. But the *relative* tail lengths (expressed as percentages of body-length) may be fairly compared, since there is good evidence that these ratios remain nearly constant after the first few months of life. The following table allows of a comparison between the two races, in respect to this character:

	Number of Cases	Mean (Percentage)	Standard Deviation
Eureka (males)	83	104.39 ± 0.37	4.95
Eureka (females)	53	103.60 ± 0.54	5.85
Samoa (sexes combined)	21	97.48 ± 0.94	6.38

The differences between the Samoa lot (sexes combined) and the Eureka males and females are 6.91 per cent. and 6.12 per cent., respectively. These differences are about seven and six times their probable errors, respectively. Their significance may therefore be regarded as fairly certain, despite the small numbers comprised in the Samoa series.

As regards foot-length, the two races do not differ significantly. But the ear, as already stated, is appreciably longer in the Samoa mice, this difference being perceptible, even without measurement. Here, as in the case of tail-length, a simple comparison of gross averages for the two groups would be unjustifiable. But in the present instance, the conversion of the absolute values into percentages of body-length would be equally unjustifiable, since the growth of the ear is not at all proportionate to that of the body as a whole. We must therefore resort to the method of "size groups," *i. e.*, we must divide each of our two lots of animals into small groups comprising individuals of nearly equal size.

In the case at hand, we have fifteen groups, or rather

pairs of groups, within which a comparison of average ear-length is possible. In twelve cases the mean figure is greater for the Samoa mice, in two cases it is greater for the Eureka mice, while in one case the two figures do not differ appreciably. The probabilities against such a preponderance being due to chance are of course high. The mean difference in ear-length between the two lots, computed according to a method described by me in an earlier paper,[7] is 0.87 mm. Those who have made careful measurements of mice will regard such a difference in the length of this appendage as far from trivial.

Let me now say something as to the environmental conditions under which these two races of *rubidus* live. Those which I have designated as the "Eureka" or "redwood" race were trapped by me during two different years, within a distance of two miles from the southern limits of the city of Eureka, California. The region is one covered in large part by redwood forest, most of which is of second growth, although there are some small areas that have never been logged. The predominant tree is the redwood (*Sequoia sempervirens*), but several other conifers are common, the most abundant of these being the Sitka spruce (*Picea sitchensis*), Douglas fir (*Pseudotsuga taxifolia*), and lowland fir (*Abies grandis*). The red alder (*Alnus rubra*), cascara (*Rhamnus purshiana*), waxberry (*Myrica californica*), red elderberry (*Sambucus racemosa*), and a willow (*Salix hookeriana*) appear to be the chief non-coniferous trees of this district.[8] The "wild lilac" (*Ceanothus thyrsiflorus*) is likewise common in some of the more open areas, often reaching the proportions of a small tree.

Except in recently cleared tracts, the region is one of dense underbrush, the shrubbery and vines forming, in fact, a veritable jungle which is frequently hard to pene-

[7] *Journal of Experimental Zoology*, Vol. 18, April, 1915, particularly, pp. 341 et seq.

[8] For the determination of many of the plants referred to in this paper I am indebted to Professor H. M. Hall, of the University of California, and to Mr. J. P. Tracy, of Eureka.

trate. Here we meet with the thimble-berry (*Rubus parviflorus* var. *velutinus*), the salmon-berry (*Rubus spectabilis* var. *menziesii*), huckleberry (*Vaccinium ovatum*), red bilberry (*V. parvifolium*), salal (*Gaultheria shallon*), and in the more open areas the blackberry (*Rubus vitifolia*). Two ferns (*Aspidium munitum* and *Pteris aquilina*) are extremely abundant, the latter in particular forming dense growths higher than a man's head. In the

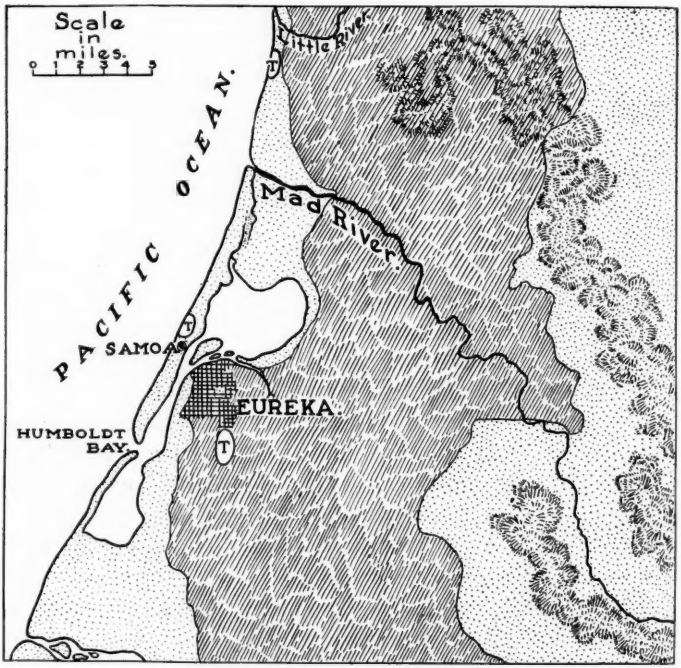

FIG. 1. Map of the vicinity of Humboldt Bay, California, based upon J. N. Lentell's map of Humboldt County. The three principal trapping stations are designated by the letter T. Area occupied by redwood forests is indicated by oblique shading.

more open areas a tall annual of the evening primrose family (*Epilobium angustifolium*) constitutes an important element in the vegetation.

One coming from the more arid parts of California can

not fail to be impressed by the prevailing humidity of both soil and atmosphere in this region. In the dense shade of the great redwoods the ground is damp, even during the summer months, and the fallen logs are covered with mosses and fungi.

When we cross Humboldt Bay to the narrow peninsulas separating this body of water from the ocean (Fig. 1), we enter a quite different environment. No redwoods are found, the woods, where present, are open, and the ground is prevailingly dry and sandy. In the wooded area, extending down the axis of the northern peninsula, the predominant tree is a small pine (*Pinus contorta*), though the waxberry and willow (*Salix hookeriana*) are likewise abundant, and small specimens of the Sitka spruce are fairly common. Among the more frequent shrubs are the huckleberry (*V. ovatum*), the twinberry (*Lonicera involucrata*) and silk tassel bush (*Garrya elliptica*). The ground is largely covered by two plants of trailing habit, the bearberry (*Arctostaphylos uva-ursi*) and the beach strawberry (*Fragaria chilensis*).

On its ocean side, the peninsula is bordered by a wide strip of shifting sand. Here the process of dune formation may be witnessed to perfection, the dunes often reaching a height of forty or fifty feet. In places the encroachments of the sand upon the hard-pressed vegetation are evidently rapid, solid ramparts of willows and spruces being steadily engulfed by an advancing wall, frequently as high as the trees themselves. Nevertheless, even on the open sands of the dunes, certain trailing plants maintain a precarious foothold. Among the commonest of these are to be mentioned the yellow sand verbena (*Abronia latifolia*), the beach strawberry (*F. chilensis*), beach pea (*Lathyrus littoralis*), and two species of Franseria (*F. chamissonis* and *F. bipinnatifida*), while the succulent *Mesembryanthemum aequilaterale* is occasionally met with.

Despite the nearness to the ocean and the high atmospheric humidity, the peninsula region seems dry in comparison with the redwood forests. This is due in part to

the loose, sandy character of the soil—where, indeed, any real soil exists—and to the comparative lack of shelter from the prevailing westerly winds. Evaporation here is doubtless more rapid than in the comparatively stagnant air of the forests.

To my surprise, the footprints of mice and other small mammals were abundant, even on the shifting sands, in the areas of sparsest vegetation. Since these tracks, for the most part, were effaced every day by the wind, the animals must have been present in large numbers. Indeed, it was in or close to the dune region that I trapped most of the twenty-eight *Peromyscus*. It seems more than possible, therefore, that the predominantly paler shade of the mice dwelling here may be due to the same causes which are operative in producing the yet paler hues of many of the desert rodents.

What the effective factors are can not yet be stated with certainty in either case. Protective coloration is of course an obvious explanation, but it is one of doubtful applicability in the case of animals which are almost wholly nocturnal in their habits. For this and other reasons it seems more likely that the pale coloration of these mice stands in some more direct relation to the humidity of their immediate surroundings. That it is not, however, a strictly "somatic" phenomenon, called forth anew in each generation, I have already shown for the desert race, *P. m. sonoriensis*.[9]

Whether or not the peculiar color of the pelage in the Samoa race is likewise hereditary I have endeavored to test experimentally. Seven living females and a number of males were brought to La Jolla in June, 1916. Unfortunately, it was not possible to obtain more than two broods of young, comprising three individuals, one male and two females. These animals were carefully examined at the age of five months, in comparison with over forty individuals, derived from the redwood stock, which were

[9] AMERICAN NATURALIST, Nov., 1915. I have since reared this race in Berkeley as far as the third (in one instance the fourth) cage-born generation, without any certain modification in color.

mainly of the same age or older, and likewise reared from birth at La Jolla. Not a single individual of the latter stock was as pale as either of the two females of Samoa parentage. The male of the Samoa race was, however, of about the average shade of the redwood descendants. As stated above, some of the wild parents, trapped on the peninsula, were likewise as dark as many of the redwood series.

No certain conclusions can, of course, be based upon these three individuals. But the condition of the two females certainly lends support to the belief that the peculiar coat color of the Samoa race, however it was acquired, has become fixed germinally.

Reference to the map shows that the northern peninsula of Humboldt Bay is largely isolated, so far as land-living rodents are concerned. In addition to the ocean and the bay, a marshy tract extends from the latter to the Mad River, which, in turn, interposes a further barrier on the north, and nearly converts the peninsula into an island. Beyond the mouth of Mad River, this same type of sand-dune formation extends uninterruptedly to the mouth of Little River, about six miles to the north, where it ends abruptly and the shore line becomes precipitous.

Now this northward extension of the sand-dune region is not isolated by any physical barrier from the redwood forest, which here comes near to the coast. It occurred to me, therefore, to attempt the collection of *Peromyscus* from a point somewhere within this region. The locality chosen was close to the ocean, about two miles south of Little River and four to five miles north of Mad River. Here the conditions were found to be closely similar to those on the exposed side of the northern peninsula of Humboldt Bay. The dunes were on the whole lower, however, and some minor differences were noted in the flora. The belt of shifting sand here ranges from five or six hundred feet to perhaps a fourth of a mile, giving place on the landward side to a narrow meadow or marshy area, succeeded by a high, steep, wooded ridge.

About ninety traps were set on two consecutive nights,

yielding in all forty-eight *Peromyscus,* all belonging to the subspecies *rubidus.* Many of these were still in juvenile pelage and such individuals were kept and allowed to mature in captivity.

A hasty comparison of the living Little River animals (as I shall call them) with those from the Samoa and Eureka trapping grounds made it plain that, in respect to color, they belonged with the latter group rather than the former. Careful comparisons of series of dead mice and of skins were made later and the bodies were subjected to the customary measurements. Owing to numerous deaths, however, only twenty-eight individuals were available for these purposes.

This more critical examination confirmed my earlier belief that the Little River mice agreed pretty closely, in average color, with the redwood stock, but that they differed widely from those taken on the peninsula. It seemed probable, however, that the mean shade was slightly lighter than that of the former animals, making them, to this extent, intermediate.

One conclusion then seemed plain. The peninsula race, exposed to certain modifying conditions, was enabled to differentiate from the mainland stock, owing to the almost insuperable barriers to migration. The Little River stock, exposed to practically the same conditions, have not formed a distinguishable race, because the rate of differentiation has been far exceeded by the rate of diffusion, or intermingling with the great body of more typical "*rubidus,*" dwelling in the redwood forests which extend back from the coast. We might seem to have, therefore, a particularly clear cut example of the effectiveness of isolation in the formation of a local race.

Now, I am not yet prepared to admit that such conclusions would be groundless. But here, as so often happens, a further study of the data has shown that the problem is more complex than was at first suspected. It is true that the mice of the more northern sand dunes have not formed a distinct race as regards *color.* But it is none the less certain that they differ from those of the

Eureka region in regard to both the length of the tail and that of the ear. In respect to the former character, they agree pretty closely with the Samoa race, the difference from the redwood stock being statistically even more certain in this case. To still further complicate the situation, we find that the ear, instead of being longer, is shorter than that of the redwood mice by about half a millimeter, and thus averages about one and one half millimeters shorter than in the peninsula race. Here, too, the differences are even more certain statistically than those which distinguish the Eureka and Samoa series.

The numbers are small, of course, only twenty-eight of the Little River mice having been available for measurement. But as regards tail length, the difference between the averages is seven to nine[10] times its probable error, so that the likelihood of its being due to random sampling is very small.

Have we, then, here merely another example of inconclusive data, which might best have been left unpublished? I do not think so. The mere existence of these local differences in color and in the size of parts deserves careful description, whatever interpretation we may place upon them.

Moreover, I am disposed to believe that the case of coat color is not entirely comparable with that of the length of the appendages. In another article[11] I have given reasons for thinking that some of the differences in the former may have arisen in nature as more or less direct effects of environmental conditions. On the other hand, I have shown that such an explanation would be of very difficult application as regards some of the measurable differences in the parts of the body, even though the latter are known to be readily influenced by various experimental agencies.

Now the evidence at hand is sufficient to show that any environmentally produced modifications of coat color are

[10] Depending on whether the comparison is made with the Eureka males or females, the sexes being combined in the case of the Little River group.

[11] AMERICAN NATURALIST, Nov., 1915.

at best rather gradual. *Rubidus* remains *rubidus* and *sonoriensis* remains *sonoriensis,* after several generations of captivity in changed climates. But even the first cage-born generation of each of my subspecies is found to be highly modified by confinement, in respect to the mean length of certain of the appendages. That this somatic plasticity would be accompanied by a high degree of germinal instability, as regards these parts, could not, of course, be predicted in advance. But the frequent appearance of local differences of type renders it probable that this is true. Whether or not these local peculiarities are due in some indirect way to environmental factors, or whether they are due to "spontaneous" mutation, need not concern us here. The main point to bear in mind is the probability that the pelage color is somewhat more stable in these mice than are the bodily proportions, despite the fact that it is the former, rather than the latter, which gives the clearest evidence of a definite correlation with known factors of the environment.

For the reason just stated, it is possible that the differentiation of a new color race might require fairly rigid isolation; whereas local differences in some of the measurable parts might arise in the presence of no other barrier than the naturally slow rate of diffusion of a non-migratory animal. As was remarked earlier, we have reason to suppose that representative collections from an indefinite number of localities would reveal the existence of statistically certain differences between the mice of many of these localities. In most cases, it would probably be unjustifiable to assign these series to distinct *races,* or other definite taxonomic groups, since it is likely that perfect intergradation would be found between most of them, and that the degree of difference would be largely a function of the distance apart of their respective habitats.

These last remarks are, of course, largely conjectural. Part of the author's present program consists in a careful study of local differences of the sort here discussed.

It is hoped that this will render possible more definite answers to some of these difficult questions.

It seems to be held by certain zoologists that any discernible difference between two local types, if at all constant, ought to be in some way recognized in the nomenclature. Indeed, I have been advised to name this modified race of *rubidus* from the northern peninsula of Humboldt Bay. Such a practise, if carried out consistently, would lead either to an endless multiplication of subspecies, or else to the introduction of quadrinomial names. Either procedure would, I think, be deplorable. The actual needs of the situation can commonly be met, I believe, by stating the locality from which a given specimen or collection was taken. The bestowing of formal names creates the false impression of a multitude of well-defined entities which do not, in reality, exist. Moreover, it is my firm conviction that nomenclature should have for its object the recognition of resemblances as well as the recognition of differences. The first of these functions is all too frequently overlooked.

SHORTER ARTICLES AND DISCUSSION

THE MIGRATION OF FISHES

UNDER the head of "The Migrations of Fish,"[1] Professor Alexander Meek has given a voluminous account of what is known of the movements and the distribution of the various families of fishes. The work is illustrated with drawings and photographs of many species, showing not only their forms and their movements, but often the stages of development and the structure of fins and scales. Especially valuable is a series of maps showing the geographical distribution of interesting groups. The word migration is taken in its largest sense, including not merely movements of individuals or of masses, but the larger problems of distribution, extending often over geological periods.

It is plain that distribution is intimately related to migration and migration to currents.

As the problems of fish conservation depend directly on the facts of migration and distribution, especial attention is given to the development and movements of food fishes and naturally to those of the North Atlantic.

After a general discussion of the continental and oceanic changes which have taken place since Eocene times, these having a direct bearing on modern conditions of fish-distribution, Professor Meek takes up the various groups of fishes, beginning with the lowest, treating of the habits, movements and distribution of each group in turn.

The excellent account of the lampreys and hag-fishes shows a certain omission. While the lampreys fasten themselves to other river fishes, sturgeons, catfishes and the like, rasping great holes with their teeth, the hag-fishes attack the throats of large sea-fishes, entering the muscular system and almost destroying it before the fish concerned finally dies. Around Monterey Bay, various flounders and rock-fishes (*Sebastichthys*) are thus attacked and drift about as living hulks while the hag-fish (*Polistotrema*) devours their muscular tissues.

The interesting parallelism in habits and distribution of the

[1] "The Migrations of Fish," by Alexander Meek, M.Sc., professor of zoology, Armstrong College in the University of Durham, and director of the Dove Marine Laboratory, Cullercoats. Edward Arnold, London, Longmans, Green & Co., New York. Price $4.50.

sturgeon and the lamprey is noted by Professor Meek and in both cases the facts now observed are of long standing. Both had apparently "refuge-regions" during glacial times. We do not, however, see the reason for the suggestion that the green sturgeon of California (now almost extinct) "may have been derived from the Atlantic during the post-glacial disturbance."

The migrations and breeding habits of the herring are treated with special fullness, proportionate to the economic value of the species. For in the north, as Björnson informs us, wherever a herring school touches the coast a town springs up, like driftwood on the beach.

In the account of the trout, very good as a whole, we may note that the genus *Salmo* is represented by different forms, originally derived from the Pacific coast, in the Great Basin of Utah, and also in the headwaters of the Colorado, Rio Grande, Arkansas and Platte, as well as the Columbia and Missouri. It is probable that the freshwater *irideus* (Rainbow trout) and the sea-run *gairdneri* (steelhead) are not really different, but both are quite separate from the cutthroat trout (*Salmo clarki*, wrongly identified at first by American authors with the Kamchatkan *Salmo mykiss*) from the Tahoe trout (*Salmo henshawi*) and from the several local forms which have sprung from these or which have preceded their advent. The last seems to be the case with the silver trout of Lake Tahoe (*Salmo regalis*). The suggestion of Professor Meek that the European salmon (*Salmo salar*) of the Miocene was divided into a North Atlantic and a Mediterranean form is interesting. The latter developed as a "trout" dividing into sea trout (*Salmo trutta*) and burn (or brook) trout (*Salmo fario*). But these two are as yet not really differentiated, corresponding in a way to the rainbows and steelheads of the Pacific coast. Professor Meek says:

> There is thus good reason for believing that the sea trout and the common trout may be the same, the one retaining the migratory habit and the other confining itself to fresh water.

Our own experience with the species lends probability to this view.

That "the salmon preceded the trouts" in time is also probable, but the western species of trout must have been derived from the trout of Europe and Asia.

The Pacific salmon must be older and more primitive than the Atlantic salmon, for the six species differ from one another, more than any trout or even the Atlantic salmon itself differs from any other black-spotted trout whatever.

Professor Meek hardly does justice to the spawning habits of the red or blueback salmon. It runs up rivers to varying distances—from one mile to 1,500 miles (Lake Labarge on the Yukon). But it never enters a stream which does not flow from a lake and it spawns always in the small streams at the head of the lake.

At Boca de Quadra in Alaska, the small stream is barely a mile long. It comes from a clear lake, perhaps five miles long. Into this stream and lake the salmon crowd by the thousands. The Yukon is not a good red salmon stream, because the nearest tributary lake, Labarge, is about 1,500 miles from the sea. Yet red salmon enter the river and reach the lake. In streams without lakes as the Skagway, red salmon are never seen. The King salmon (*Oncorhynchus tschawytscha*) also runs for great distances, but it is absolutely indifferent to the presence of lakes. It is probable that the red salmon spend their first winter in the lake and some never leave it, remain landlocked and dwarf until spawning time (usually four years).

One of the most difficult of problems is to understand the instinct of the red salmon. Every individual of this and of each of the other species of Pacific salmon (*Oncorhynchus*) dies after spawning. How does the spawning fish, stupid in most regards, know when it enters a river that there is a lake before it? How does it come to avoid all lakeless tributaries as it goes up, finally reaching the lake's head and the brooks that feed it? And why do the other salmon species totally lack this instinct? There are other problems, yet unsettled, regarding the supposed homing instincts of salmon. The majority (but not all) seem to return to spawn to the parent stream which they left as fingerlings. Why not all? And why any?

The recognition of the age of salmon and trout by the adjustment of the rings on the scales, as recently worked out by Dr. C. H. Gilbert and others, received full attention from Professor Meek. The scales of the salmon are marked by concentric rings of growth, and these are more widely separated in the summer, the feeding time of the salmon when the individual grows most rapidly.

Professor Meek devotes much space to the singular breeding habits of the eel, which spawns in the sea, entering rivers to feed. But many individuals, in our Mississippi Valley never descend to the sea. A very large eel, once taken by the present reviewer, above the Cumberland Falls in Kentucky, 2,000 miles from the

sea, must either have never spawned or cast its spawn into the river.

The larvæ of eels as well as of some other soft-rayed species are quite pellucid, without pigment cells and with "a roomy space between the skin and the muscles, distended by a watery fluid." Many of these larvæ, in their transformation to the condition of young fishes become much reduced in size, though increasing in weight, by the obliteration of these interspaces.

Professor Meek's studies pass through the whole long series of fish-families. For want of space, we may not follow them further in these pages. We must give the work, as a whole, very high praise as carefully, intelligently and scientifically done, and as constituting a reference book of great value. The author has well covered the range of the periodicals which treat of the distribution and habits of fishes. He seems, however, to have overlooked the most extensive recent work of a similar range, "Jordan's Guide to the Study of Fishes," published in New York in 1905.

DAVID STARR JORDAN

NEW LIGHT ON BLENDING AND MENDELIAN INHERITANCE

UNDER the above heading, Dr. Castle reviews a paper by Yuzo Hoshino on the inheritance of the flowering time in peas and rice.

Since reading this review, Prof. Hoshino kindly sent us his paper, and we have ourselves examined it with care to see whether indeed it necessitates Dr. Castle's rather sweeping conclusions, namely, that certain genes are themselves modified by crossbreeding, one of the conclusions of Hoshino himself, and that selection within a pure line, within a genotypically pure population is effective.

It is well known that Dr. Castle counts among the few last geneticians, who still believe that the genes themselves are modifiable by selection. Hitherto in nearly all his writings on the subject Dr. Castle claimed, that unit characters vary, and may be modified by selection, a statement which can not very well be opposed, given the loose way in which the obsolete term unit character is usually applied. But it was clear, that Dr. Castle really believed the genes themselves to be capable of variability in potency, quality and value, and we think it of the utmost importance that in the review under discussion he has stated the

question in these words. Thus the issue between Dr. Castle on one side, and Johannsen and us on the other narrows, and there need be no more difficulty as to the exact meaning of the term unit character. As to the effectiveness of selection in genotypically homogeneous material, all the evidence so far adduced shows that selection in such material is absolutely ineffective. It is evident that selection in a population is usually effective, but this only shows that in ordinary populations, even in so-called pure strains of animals, there is a good deal of genotypic variation, or in other words impurity.

The fact, for instance, that Dr. Castle's selection in hooded rats was effective, shows that his material was not originally pure for all the genes. In all those instances where the guarantee for genotypic purity of the material was reasonably good, selection has, until now, proved ineffective. We need only point to the fifty years of selection in wheats by the de Vilmorin family, and to the numerous selection-experiments with clones of *Paramœcium* by Jennings and others.

As to the so-called instances of the effectiveness of selection on the genes themselves in alleged genotypically homogeneous animal material, we repeat that the only way to show such an effect in material which offers no sure proof of purity would be to change a strain of severely inbred animals by selection to a point removed from the range of the ordinary modification in the material, continuing the inbreeding, and then, by contraselection, to bring the character under consideration back to its starting-point. Since we wrote down this challenge to the believers in the variability of genes, one such a series of selection-experiments has been performed, namely, on flies, and in this series it has been proved to be impossible to get the material back to its original quality.

According to Dr. Castle, Hoshino's Table 6 shows the effect of selection within a pure line. In the cases taken from Hoshino's paper, in which the progeny of an early-flowering and a late-flowering individual of the same "pure line" can be compared (in the original table there is one more case in which the earliest parent gives the latest progeny) there are more instances in which an early parent gives an earlier progeny than a late parent, than cases in which an earlier parent gives a later progeny. But if we examine the figures more closely, we observe that the mean deviation of offspring from parents in the case in which the earlier parent gives the earlier progeny is 0.52 day,

whereas the mean deviation of offspring from parent in cases in which the earlier parent gives the later progeny is larger, 0.65 day. The only conclusion from Hoshino's Table 6 is the one he makes himself, namely, that the variation is insignificant.

We are sure that no unbiased person would conclude from the negative facts in the table in question that the variation in these pure lines was genotypic, or that selection in these groups has had an appreciable effect.

On page 332 Dr. Castle writes:

> If I have correctly interpreted Hoshino's observations, flowering time in peas is clearly a Mendelian unit character, entirely devoid of dominance, so that a strictly intermediate hybrid form is the commonest end product of a single cross between early and late varieties.

Indeed, if Hoshino's work on the inheritance of flowering-time of peas were the only, or the first, or the most comprehensive of its kind, we could see reasons for such a belief. But Hoshino only crossed two varieties differing in time of flowering. But in peas there do not exist only one late and one early variety, but several thousands, each with its own time of flowering. It would not be difficult to give a list of ten names of pea-varieties of which in every preceding one all the plants would be in flower before one of the next opened its first flower. Therefore crossing experiments involving two varieties can never be sufficient basis from which to conclude that flowering-time in peas is one thing or the other.

Tschernack, in his well-known experiments with flowering-time of peas (1911, Mendel's Festchrift), cited by Hoshino, made eight different variety-crosses. Whereas in Hoshino's work the two varieties crossed happened to be of such a constitution, that in the resulting F_2 generation there did not occur plants which commenced flowering at an earlier time than the earliest parent, or at a later date than the latest parent, in Tschernack's work such cases were met with. In Tschernack's experiment No. 81 (1906) there were in F_2 found plants flowering seven days earlier than the early parent; in experiment No. 82 (1916) even plants beginning flowering nine days earlier than the early parent. In experiment No. 81 (1906) there were also found plants starting to flower four days after the latest parent, and in experiment 38 (1902) there were plants, which did not begin to flower before the late parent had been in flower for a week.

It is perfectly clear, that a sort of blending may be the result of a difference between the parents in a number of genes, influencing the quality under observation in different directions.

On page 333 Dr. Castle writes:

> In typical blending inheritance the determiners of contrasted parental conditions apparently blend into a determiner of intermediate character, the gametes formed by an F_1 individual being practically as uniform in character as those of either parent. Blending is illustrated in the inheritance of ordinary size-differences in birds and animals.

No one who knows the work of Punnett and Bailey (cited in Hoshino's paper) on chickens, in which they found not only individuals in F_2 as small as the smallest parent and as large as the largest, but even individuals lighter than the lightest parent and heavier than the heaviest, could maintain that ordinary size-inheritance in birds is blending. The gametes formed by the Hamburg $\times$ Sebright hybrids, or by our Leghorn $\times$ fighting bantam certainly were not as uniform as those of any of the four parental strains!

We are perfectly in accord with Castle when he reasons that if once we admit a contamination of genes and qualitative changes in genes, we do not need to assume that flowering-time in peas is influenced by two genes, in the cases studied by Hoshino. In such a case the difference in one gene would suffice. Indeed, we would go one step farther than Castle and declare, that, on the assumption of qualitative changes in genes, we need not assume a genotypic difference between the parent varieties at all. Where we differ from Dr. Castle is in the fact that we do not believe in qualitative variation of genes. Surely more than ten genes must influence the beginning of flowering in the pea, else there could not be so many varieties differing in the time of flowering. All the genes which influence stature, shape of flowering axis, color, must necessarily influence the onset of flowering. And we need not look for coupling between color factors and flowering-time factors, because the factors influencing color influence the metabolism of the whole plant, and thus the period at which it starts flowering.

If we compare Hoshino's paper with Tschermack's extensive experiments on the subject, we find nothing in it, which would make us assume contamination of genes by crossbreeding, or any qualitative variability of genes.

A. C. HAGEDOORN,
A. L. HAGEDOORN

NAGASAKI,
December 19, 1916